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THE CELL—SOME ASPECTS OF ITS FUNCTIONAL ONTOGENY[1]

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BIOLOGY, like all material things, must of necessity change and, indeed, a study of its history seems to indicate certain slow but definite and well-marked trends. Some of these, on first inspection, have seemed radical in nature but through the seasoning effect of time they have proven but a part of a slowly moving panorama of events. Fads and fancies have sprung up—they have grown, withered and in many cases died, but the fundamental and basic threads of an enduring biology continue. As the world of affairs quickens its pace and tempo, just so the intensity and capacity factors seem to rule and govern the program of scientific research and discovery. It has been well said and even to-day actually demonstrated, that we live in a rapidly changing world; the results of such gigantic movements seem too great and ponderous for immediate digestion and interpretation. Biology, as an integral part of things, has at times in its history seemed almost as uncertain and confused as are the modern trends of human affairs. In general, we like to believe our modern concepts and ideas in experimental biology represent advances over those of our too often forgotten predecessors. Such a fallacy, however, becomes immediately apparent if we but carefully analyze the meager array of basic and

[1] Address of the president of the American Society of Naturalists, Philadelphia, January 1, 1941.

fundamental results added to those long since handed down or suggested to us. To be sure, we too often boast of a different and more exact experimental approach and also perhaps of a regrettable scorn for the older morphological (?) techniques, but the cold fact remains we have added but little of real fundamental and basic information. We have passed from what might be termed a static to a more or less dynamic view-point, but our supply of really pertinent and fundamental biological information is still extremely meager. We pass from a "cell-theory" to one of proven fact, but the implications of either from a strictly basic or fundamental point of view seem as far from reality as ever. We too often have been prone to dress up an old model or form with a new set of terms with the hope that the new dress will tell us more of the nature of the model itself. However, there are from such dilemmas inviting avenues from which new vistas appear and which afford stimulus to increased enthusiasm and determination for future concerted efforts in attempts to push the borders of biological knowledge to further horizons. The "wanderlust" of the experimentalist, the dreamer if you please, is the very life blood upon which future developments in biology rest and to which we must all look for inspiration so necessary for the molding of our future. The history of biology is, to be sure, but a history of the thoughts and ideas of the persons involved; its future to the same degree must rest upon the ability of workers to think and act—to think clearly and to act wisely.

During the past few years biologists have turned their attention toward solutions of problems in so many and diversified fields that it is difficult to appraise the work of the staff of the modern experimental laboratory in pre-existing terms. We hear newly coined phrases—biophysics, borderline fields, and a host of others. Surely the modern biologist seems to have spread his attentions in many directions in attempts to find methods and tools suitable for the better measuring of biological phenomena. Where and what the end will be has given many great

concern. Never before have such refined and varied working tools been available to the experimental biologist. What can the future hold in store? We ask ourselves this question—the answer seems to be still far in the distance.

With this brief and scanty appraisal of present situations and the future for biology well in the foreground, I should like to speak more specifically and in some detail concerning a field of endeavor which may in some small way illustrate what can be done by use of the relatively "simple" in attacking various phases of certain fundamental and basic cellular problems still confronting the biologist.

To such an audience one needs scarcely to attempt to point out the so-called "practical" sides of fundamental or basic types of biological investigations. However, there seems at times much to be gained from a review of the methods and results of so-called "practical" experimentation since the simple and more obvious gaps in our knowledge of fundamentals is most clearly accentuated there. A very striking example of this can be had from the great amount of effort expended in studies on malignant types of cells. A no more difficult field of endeavor has been so courageously attacked but with it all how greatly handicapped the workers have been without information regarding the fundamental workings of the so-called normal embryonic cell. How fruitless and expensive it so often has been to spend long periods of study endeavoring to learn of the "sick" cell only to eventually discover that concerning the "healthy" cell so little is known. With such a background and fully conscious of the intense study demanded, it has been possible to make a feeble beginning in an attempt to learn something of the workings of the normal embryonic cell, and it is concerning these results I should like now to direct your attention.

Without burdening you with technical details or methods of procedures, I should like to outline with you the data which I hope will convince you that relatively simple

biological materials are readily available and which may be standardized and made useful in almost any of the important and basic problems in cellular biology. As you doubtless are already aware, any experimental biological program depends for its success upon the choice of biological materials which are readily available—are easily standardized—are relatively simple and above all lend themselves to experimental techniques. Arguments pro and con for intensive concentration on a single type form or upon a varied number of animal forms can be well advanced. In the present work, the egg and embryo of the common grasshopper, *Melanoplus differentialis,* have been extensively employed. After some years of intensive study it is now possible to raise these animals in the laboratory so that a large daily supply of fresh eggs is always available. The costs of such operations are as low if not lower than for any experimental animal thus far employed on a large scale in biological work. Much detailed information has been gained concerning this form, and I believe it would be quite fair to state that as much accurate quantitative data are now available for it as for any other biological material. Due to the extensive cytological work of Professor C. E. McClung and his students on this group, we have at hand detailed information as to the nuclear and other cellular structures of many different species of this group. These cells are large, easily handled in a technical way and have shown themselves to behave in a cytological fashion typical for any animal cell. Both parthenogenetic and fertilized eggs are available for study, and some of the most interesting chromosome behavior in parthenogenetic development has been elucidated through cytological studies of such cells. Problems relating to developmental blocks occasioned or produced as the result of haploid-diploid chromosome conditions can most easily be attacked by use of such materials. Studies on the formation of the egg and embryo along with their related structures have yielded much of general as well as specific information, *e.g.,* the formation of the egg membranes, the part

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played by yolk and serosa cells, the development and action of the so-called hatching enzymes, the hydropyle, and a host of others are but general questions about which much information has been gained.

In any quantitative cellular study it is fundamentally important and necessary to first establish a time-table of events under as near standard environmental conditions as possible. Such a table of developmental activity at 25° C. has been worked out for the grasshopper from the time of fertilization to the hatching of the embryo. It is now possible, therefore, to give an accurate and timed picture of the morphology and to a lesser degree the physiology of the egg throughout its entire course of development. The advantages of such a material for experimental procedures are too numerous to mention except to emphasize the satisfaction that comes from being able to experimentally reproduce results time and time again.

With such a standardized material an intensive study has been made of the physiology of the developing cells and embryos and it is about certain phases of these investigations I should like to talk with you in some detail. Being a cold blooded animal, development of the egg of the grasshopper depends for its rate more or less directly upon the surrounding temperature. When kept at 25° C. the eggs develop in a regular manner for a period of three weeks, at which time they cease development and enter into a developmental rest or block (diapause). This block is a general one extending not only to the cells of the embryo proper but also to the yolk, serosa and other cells of the egg. If left at 25° C. the duration of this block varies from a relatively short time to even a year or more. Experimentally, an exposure of the blocked eggs to 0–5° C. for appropriate periods completely destroys the inhibitor so that when again placed at 25° C. development again proceeds at a normal rate up to the time of hatching. Occasionally few eggs are obtained which do not seem to possess the inhibitor and when kept at 25° C. no changes in developmental rates appear. The fundamental significance of diapause or developmental block for cellular

physiology is that it makes available a natural situation in which normal embryonic cells may be studied both when in active mitosis and growth as well as when in a relatively inactive state. Several physiological phenomena have thus been studied but due to the time here allowed but one or two will be discussed in some detail.

Among the methods for evaluating the general metabolic conditions of cells their rates of oxygen consumption and carbon dioxide output seem to offer a fair degree of promise. Detailed studies have, therefore, been carried out on the respiratory activity of developing eggs both in their actively developing and blocked phases. Changes in rates of cellular activity seem to be definitely correlated with respiratory activity; when actively growing and dividing, the rates of oxygen uptake are high, when blocked these rates drop to low values and remain so throughout such periods. Further study has revealed that the respiratory mechanisms in the two physiological states of these cells differ. In the actively dividing and growing cell, we find a carbon monoxide-cyanide sensitive-type of respiration while in the blocked condition, we have a carbon monoxide cyanide-insensitive type of respiration. The real significance and importance of this difference is at present not clear, but it is of interest to note that in certain abnormal and pathological cells strikingly similar situations occur. Closely correlated with these differences in respiratory mechanisms between active and blocked cells are such phenomena as; marked changes in sensitivity to x-irradiation, striking differences in the affinity for the retention of water, marked differences in response to reagents such as methylene blue, di-nitro phenols, etc.

A detailed study of the respiratory quotients shows that types of food utilized during development vary for different periods, indicating, perhaps, that the energy of development must in some way be closely correlated with the fundamental behavior of the developing cell.

Among the problems of immediate interest are those relating to the origin and modes of functioning of certain intra-cellular enzymes. Such studies become increasingly

important in view of the fact that practically nothing is known in a quantitative manner concerning the origin of intra-cellular enzyme systems. A review of the literature as well as our own personal concepts would often lead us to believe that the cell when formed is chemically and otherwise complete. This idea, however, seems rather fallacious especially as regards intra-cellular enzyme systems. The development and mechanism of action of the color- or melanin-producing enzymes have been rather intensively studied in the grasshopper egg. These enzymes, as you doubtless are aware, belong to that group, designated *oxidases* and the specific one with which we are at present concerned, *tyrosinase*. Due to the current interest in the questions of color and melanin formation in developing cells and organisms it becomes of added interest to inquire into the ontogeny and modus operandi of these enzymes. By means of ultracentrifuge techniques, it is now possible to stratify the contents of living eggs and cells by high centrifugal force. With such techniques, the egg of the grasshopper after centrifugation forms into three main layers. (1) A thin oily or lipoidal layer; (2) an aqueous protein-containing layer; and (3) a denser layer of inert cellular constituents. By rapid separation and removal of these layers, it can be shown that the melanin-producing enzyme is found exclusively in the middle or aqueous layer. When so separated, it is further found that the enzyme as such is in an inactive form, *i.e.*, when added to appropriate substrates no activity can be detected. In order to put this inactive enzyme, pro-enzyme if you will, into the active state it seems necessary to add to it the top, oily or lipid layer obtained from centrifugation. When this is done and appropriate substrate added the pro-enzyme becomes activated and with the oxygen of the air produces the characteristic black or melanin. The presence of both the inactive enzyme (pro-enzyme) and its activator from freshly centrifuged eggs permits one to study not only the ontogeny of the enzyme and the activator but also the possible mechanisms by which activation is brought about. Inactive enzyme or pro-enzyme preparations of high

degrees of potency and purity can now be satisfactorily prepared. It has thus been possible to follow the development or ontogeny of the enzyme throughout its course of development. No enzyme can be detected in the egg until approximately the tenth day at 25° C. From this time on the enzyme grows in an autocatalytic fashion, reaching its maximum concentration at about the fifteenth day. It then remains constant throughout the remainder of the egg's development. At all ages of the egg, when obtained by the centrifuge technique, the enzyme occurs in the inactive or pro-enzyme state. Growth of the lipid or natural activator containing layer has also been studied in a similar fashion. The potency of the naturally occurring activator is highest during the early development of the egg, indeed, even before the formation or presence of the pro-enzyme. Its potency gradually falls to the diapause period when it remains constant during this period of cellular block. When the inhibitor or diapause-producing factor is removed the potency of the activator gradually diminishes up to the time of hatching. Curiously, the potency of the naturally occurring activator is always great enough to more than activate all the contained pro-enzyme. We thus have in the developing egg the natural activator present from the beginning but decreasing in potency with development; on the other hand, the pro-enzyme occurs from the tenth day of development on to hatching. Another essential link in any enzyme system is the substrate upon which the enzyme works. In the present system no appreciable amounts of naturally occurring substrates are found until very late in the development of the egg, during post-diapause. It is thus evident that in this particular enzyme system the appearance or growth of the enzyme, its activator and its substrate is not simultaneous but rather follows a more or less specific course.

Since the melanin-producing enzyme can thus be isolated in its inactive or pro-enzyme state and its naturally occurring activator also obtained, it then becomes of rather marked interest to learn just what activation of the pro-enzyme is and how it is brought about. Not only

is this question of importance for the production of melanin but it may be of equal and if not of greater significance in aiding in the elucidation of the action of intracellular enzymes in general. As a matter of fact, it can be shown for this enzyme system that a definite "activation-inhibition" system exists within the normal cell.

Activation of an intra-cellular or any other enzyme has been and still is an extremely complex physico-chemical problem. Much theory and but few facts exist in the literature. Results of studies on the mechanism of activation of pro-tyrosinase as obtained from the grasshopper egg will be briefly discussed in the hope that some light may be shed upon possible biological relations of such phenomena. In addition to the naturally occurring activator of pro-tyrosinase, it has been found possible to bring about the same results by various physico-chemical reagents, *e.g.*, chloroform, urethane, urea, heat, detergents (aerosol, dupanol), etc. Most of these reagents are definitely known to denature proteins and some are markedly surface active. No differences in the properties of the pro-enzyme when activated by these various reagents are found; the end result is the same—*active enzyme*. The enzyme, tyrosinase, is protein in nature and as such shows all the characteristic properties of this chemical group of compounds. It, therefore, would seem logical to expect that activation may possibly be related to or dependent upon some physico-chemical properties of this protein molecule and that the various activators employed bring about just such changes. Without adding unnecessary detail in the way of experimental data it may be stated that activation of the melanin-producing enzyme in the present studies is thought to be brought about by the selective adsorption and orientation of the pro-enzyme molecules by the activating reagents. Such adsorption and orientation is already well known for other physico-chemical systems. When pro-enzyme is brought into contact with appropriate activators, definite quantitative relations seem to exist between the amounts of activator and pro-enzyme employed. When the surface of the activator has been covered or saturated with pro-

enzyme molecules no further activation seems possible. Additions of more activator produce more active enzyme and such action continues until all enzyme is adsorbed. Further addition of pro-enzyme after the activator surfaces are saturated or covered produces no more active enzyme. If such a surface concentration of pro-enzyme molecules exists on the activator then it should be possible to test and check such a situation in various simple ways. Other protein molecules than pro-enzyme ones can be adsorbed upon the activator surface and completely cover or protect it and when thus placed the pro-enzyme molecules can no longer be adsorbed or activated. If other such protein molecules could be shown to exist in the living cell and at the same time were to become adsorbed or concentrated upon the surfaces of the naturally occurring activator, then one might well visualize a system whereby both activation and inhibition in the living cell might take place in respect to the activation of the enzyme system under consideration. That such a possibility does actually exist has been experimentally verified not only by the use of proteins derived from the living cells themselves but also by the use of laboratory purified proteins. It, therefore, seems reasonable to assume that perhaps natural inhibition of intracellular enzymes may function after some such fashion.

The significance of such an activator-inhibitor system for the melanin-producing enzyme is perhaps not so important in itself, but when viewed from the normal working of the living cell, it gives us a reasonable and possible physico-chemical method which may be used in the explanation of the workings of many cellular phenomena. Problems in melanin production and the inheritance of same may well be restated in terms of such an activator-inhibitor system. And finally it would seem from a rather intensive review of the literature on enzymes and their actions that this activation of the melanin-producing enzyme seems to be the first genuine case of activation taken directly from the living cell without the usual drastic chemical treatment.

EXPERIMENTAL CONTROL OF DEVELOPMENT AND DIFFERENTIATION

GENETIC CONTROL OF DEVELOPMENTAL REACTIONS[1]

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DURING the last forty years the mechanism of the transmission of hereditary characteristics has been elucidated by the work collectively referred to as classical genetics. With the gradual unfolding of the rules of this "geometry of inheritance" the concept of the gene as the hereditary unit has taken form. And through this concept, geneticists, biochemists, physiologists and embryologists are coming to realize that only through the agency of a united front can they arrive at a fuller understanding of the nature and causes of development. We take it that the holding of the symposium of which this paper is a part is itself recognition of a faith in the profitableness of such cooperation.

The development of a relatively undifferentiated single cell into an integrated functioning organism may be thought of as the gradual elaboration of synchronized structures and functions through a network of processes that must ultimately have its basis in many temporally and spatially interrelated chemical reactions. The kinds of reactions and their interrelations will both determine the nature of the organism. Since the nature of the organism depends on the properties of the genes in the undifferentiated cell from which it develops, it may be assumed that genes in some way control the reactions both as to their kind and their time relations. Just as the organism may be thought of as an integrated whole, so the complement of genes that regulate its development may be

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thought of as a whole or unit of higher order. This conception of the interaction of all the hereditary units was early arrived at by geneticists. It does not, however, lead to the necessity of abandoning the gene concept as Goldschmidt has recently argued.

What are the modes of attack by which we can hope to learn more about this genetic contol of developmental reactions? Since at present genes of higher organisms cannot be isolated, even collectively, a direct approach is obviously not possible. If, however, as several biologists have suggested, viruses should prove to be essentially primitive naked genes, it may well be that the remarkable recent advances in our knowledge of the nature and structure of these highly organized proteins may at the same time represent revolutionary advances in the field of genetics. The relation between genes and enzymes also may prove to be direct enough to bring the rapidly developing field of enzyme chemistry into fruitful collaboration with the fields of genetics and embryology. Hopeful beginnings in this direction have in fact already been made. In a similar way immunogenetic work may lead to relatively direct means of getting at the nature of gene action.

The study of development itself is, in one sense, merely another aspect of the basic problem of how genes do their work, but in general it is probable that the phenomena with which experimental embryologists concern themselves are relatively complicated in terms of gene action. The truth of this is well illustrated by the work of Poulson (1940) on the genetic control of early embryological processes in Drosophila. The absence of blocks of X-chromosome genes result in disturbances of such magnitude that in many cases the processes of embryogenesis entirely fail to get under way. In general, reactions which may conveniently be called terminal are the ones most likely to be modified by non-lethal mutant genes, and accordingly the conscious selection of such reactions for study would appear to offer the greatest hope of relat-

ing genes and reactions. A number of examples of this nature are known. Bernheim has shown that the pigment responsible for the yellow fat in a mutant type of rabbit is xanthophyll. In normal light-fatted rabbits this xanthophyll is destroyed by oxidation, and it is assumed that its accumulation in the yellow-fatted mutant is due to lack of a xanthophyll oxidase. This enzyme in turn is presumably gene-controlled. The failure of alcaptonuric individuals to oxidize homogentisic acid (Garrod and others), and the incomplete transformation of uric acid in the Dalmatian coach hound evidently represents similar non-vital reactions controlled by genes. Pigments such as the anthocyanins of plants and the melanins of animals are substances which, though they may play significant rôles in the economy of the organisms that elaborate them, are non-essential in the sense that the organisms can develop and live more or less normally without them. The work of Onslow, the Robinsons, Scott Moncrieff and others (see review by Lawrence and Price, 1940) on the chemistry and genetics of anthocyanins indicates in a remarkable way the rôles genes may play in controlling specific reactions. In general, pigments may be expected to be useful in the study of gene action both because chemical study of them is often relatively simple and, since they are often non-essential to the life of the organism, many non-lethal genes are known to control their production and chemical properties.

We shall confine our remarks largely to the eye-pigment system of Drosophila. Although the chemistry of the pigment components concerned here is not well understood at present, the genetic control is probably better understood than that of any other comparable developmental system.

The red color of a wild-type Drosophila eye is due to the presence of two primary pigment components, a fact first fully appreciated by Mainx. There is a red, water-soluble pigment that acts as a pH indicator. This pigment is yellow in acid and red in alkaline solution. It may be

reversibly reduced to a colorless compound. The second component, a water-insoluble brown pigment may also be reversibly oxidized and reduced. It is yellow in the oxidized state and red in the reduced. Apparently in the wild-type fly both oxidized and reduced forms of this component are present in equilibrium.

Both the relative and absolute amounts of these two pigment components are subject to the genotype of the animal. In addition the equilibrium between oxidized and reduced brown pigment appears to be gene-controlled. Mainx has reported that in sepia flies the red pigment is qualitatively modified to what we shall refer to as "sepia pigment." This modification is, according to Mainx, gene-controlled by way of an enzyme system.

As examples of the manner in which genes may control this pigment system, it is known that certain recessive alleles of the genes, vermilion (X chromosome), cinnabar (second chromosome), scarlet (third chromosome) result in the absence of the brown component, *i.e.*, the normal alleles of these genes are necessary for the formation of a full amount of brown pigment. The absence of brown pigment which results from the substitution of recessive alleles for the normal alleles of any one of these genes, leaves only the red component and results in a bright red pigmentation of the eye. The recessive alleles of karmoisin (third chromosome) and cardinal (third chromosome) result in less than the normal amount of brown pigment and accordingly give an eye color more or less intermediate between wild type and the bright red of vermilion, cinnabar or scarlet. The brown gene (second chromosome), on the other hand, controls the production of red pigment. In the presence of recessive alleles of this gene, with other genes wild type, the red pigment is absent, and the eye is accordingly phenotypically brown. The white gene is concerned with the production of both pigment components and in the presence of the ultimate recessive alleles of this gene, no pigment at all is formed in the eye. For an appreciation of these relations we are

indebted to the work of Schultz (1935), Mainx (1938), Becker (1939) and others.

The studies of Sturtevant on mosaics made up of vermilion and non-vermilion tissues (gynandromophs) first suggested that the formation of the brown pigment component is dependent on some diffusible substance moving from one part of the body to another. By the use of the transplantation technique, Ephrussi and Beadle were able to confirm this conclusion and to further show that a second diffusible substance intervenes in the formation of the brown pigment. One of these, v^+ substance or hormone, is controlled by the vermilion gene and the second, cn^+ substance or hormone, formed only if the first is present and presumably derived from it, is dependent on the cinnabar gene. The sources of these two hormones are known; v^+ substance is formed by Malpighian tubes, fat bodies and by eye tissue itself, while cn^+ substance is formed only by Malpighian tubes and by eye tissue.

Although superficially the eye-pigment system of the meal moth Ephestia appears to be different from that of Drosophila, there is now evidence of so many parallels in its development and genetic control that we may assume it to be essentially similar (see reviews by Kühn, 1937, Becker, 1938, and by Plagge, 1939). In this insect, normal eye pigmentation is under the control of a diffusible substance that seems to be interchangeable with the v^+ hormone of Drosophila. Here, however, the number of known mutants affecting the color of the eyes is more limited than in Drosophila. For this reason, no evidence of a hormone corresponding to the cn^+ substance of Drosophila can be obtained, although by analogy there is reason to suppose it to be present.

It was, of course, recognized early in the work with diffusible substances involved in eye-pigment development that if we were to push the analysis very far, it was necessary to learn more about the chemistry of both the diffusible substances and of the final pigment whose presence they control. It was shown by Ephrussi and Harnly

(1936) that the hormones can be obtained in cell-free lymph. They can be obtained in amino-acid containing fractions from Drosophila or Calliphora pupae (Khouvine, Ephrussi and Chevais, 1938; Tatum and Beadle, 1938). Isolation of this active material from fly pupae, however, has been unsuccessful up to the present time.

Because of the difficulties of isolation in pure form of hormone from wild-type pupae, an indirect approach to the problem has been followed and has led to some success. Khouvine, Ephrussi and Chevais (1938) found that under certain levels of nutrition the presence of tryptophane in the food led to a partial restoration of brown pigment to a vermilion fly which under normal conditions has practically none of this component. In following up this lead, Tatum (1939) found that under aseptic conditions tryptophane was without effect in restoring pigment to a vermilion fly, but that in certain bacterially contaminated cultures it did result in the production of brown pigment. Isolation of the contaminant gave a culture of Bacterium sp. which when grown aerobically on a medium containing tryptophane produced a relatively high yield of a substance with v^+ hormone activity. Isolation of this in pure crystalline form proved to be simple (Tatum and Beadle, 1940). Before analyses had established the chemical nature of this material, Butenandt, Weidel and Becker (1940), on the basis of the work mentioned above on bacterial synthesis of the active material from tryptophane, were led to try known intermediate products of tryptophane metabolism. They found that kynurenin had v^+ hormone activity in both Drosophila and Ephestia. We have confirmed this finding for Drosophila, and Tatum and Haagen Smit (unpublished) have found that the active material made from tryptophane by the bacterium is kynurenin combined with sucrose. Hydrolysis removes the sucrose and leaves the activity unaltered.

An indication of the activity of kynurenin can be had from the fact that 1/20,000 cc of a one per cent. solution of the kynurenin-sucrose combination injected into a 60-

hour-old vermilion brown Drosophila pupa will result in the formation of a visibly detectable amount of brown pigment within 20 minutes. The final pigmentation corresponds to that of about 20 units of hormone on the scale devised by Tatum and Beadle. Expressed in another way, 1/80,000 of a milligram of kynurenin will produce a marked effect on pigmentation when injected into a single pupa.

Unpublished work (Tatum), the details of which can not be presented here, is consistent with the assumption made by Butenandt, Weidel and Becker that the natural fly hormone known as v^+ substance is identical with or closely related to kynurenin. Kynurenin can be transformed into cn^+ hormone either by the fly or by a microorganism.

In an attempt to summarize and indicate the interrelations of the facts outlined above we have drawn up a partly hypothetical scheme (Fig. 1). In this, tryptophane is shown as the precursor of kynurenin (v^+ hormone), with the transformation under the control of the vermilion gene. Kynurenin may be transformed either to kynurenic acid, which is inactive, or to cn^+ hormone which is in the chain of reactions leading to brown pigment. We suppose these alternative transformations of kynurenin to be competitive. In a wild-type fly there is presumably enough kynurenin so that both reactions may take place. In a vermilion fly, on the other hand, kynurenin is produced in much smaller amounts and the greater part of this is inactivated by oxidation to kynurenic acid. (A small quantity of kynurenin is evidently transformed into cn^+ hormone in a vermilion fly since a very small amount of brown pigment is normally formed.) We suggest that the suppressor of vermilion gene may control the oxidation of kynurenin to kynurenic acid. In the presence of the recessive alleles of this gene in an otherwise vermilion fly, kynurenin is restored because the competing reaction leading to kynurenic acid is blocked. Reduction of oxidative metabolism such as

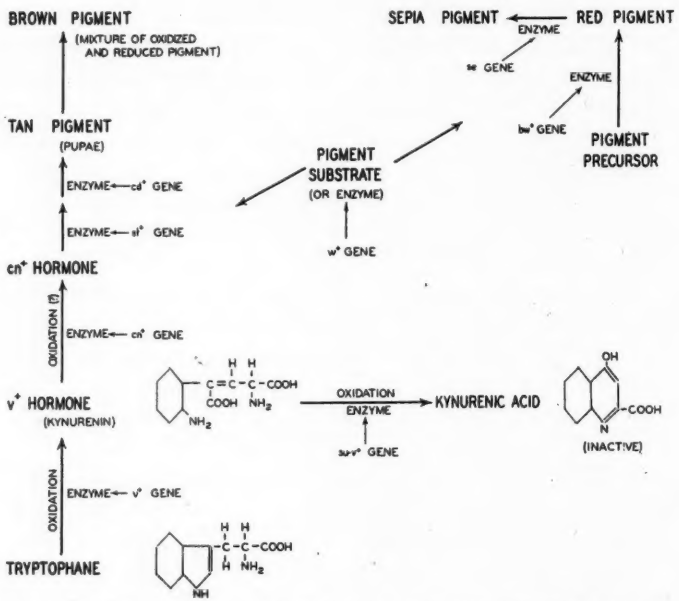

FIG. 1. Scheme of assumed steps in the formation of eye pigment in Drosophila. See text for explanation.

occurs when larvae are fed on a limited amount of food deficient in carbohydrate (Rudkin, 1939), presumably acts in much the same way (so-called starvation effect on vermilion). The transformation of v^+ hormone to cn^+ hormone, probably also an oxidative process, is controlled by the cinnabar gene. In the presence of cn^+ hormone, a reduced amount of brown pigment, or none at all, is formed if the cardinal or scarlet mutant genes are substituted for their normal alleles. The nature of the steps between cn^+ hormone and brown pigment is at present unknown, but Ephrussi and Chevais (1938) have shown that in the production of eye pigment eye-color hormones (we suppose cn^+ hormone) are used up. The reactions responsible for the utilization of cn^+ hormone in the formation of pigment appear to be under the control of the cardinal and scarlet genes. On its first appearance in the pupa, the brown pigment is largely or wholly in the

oxidized state and as development continues, the equilibrium shifts in the direction of the reduced form. It seems probable that a continued shift in the same direction after emergence may account for the age-change phenomenon in so far as the brown pigment is concerned.

Our information about the development of the red pigment of the eye is more limited than is that of the brown. It is clear, however, that in its production some reaction under the control of the brown gene is involved.

Since a fly in which the ultimate recessive alleles of the white gene are present lacks both red and brown pigments, we can infer that in the development of these two pigments there is a common step. This inference is based on our faith in the unproved assumption that a given gene has a single primary action. It may be that the white gene is concerned with the formation of a substrate common to the two pigments, or it may control the production of an enzyme catalyzing basically similar reactions in the two parallel reaction chains. The effects of the suppressor of vermilion gene also suggest a common step in the formation of the two pigments. In the presence of the vermilion gene, the recessive alleles of the suppressor of vermilion gene results in the formation of brown pigment, and this is brought about through the restoration of v^+ hormone. The suppressor of vermilion gene is also capable of suppressing purple. Here the suppression is largely brought about through an augmentation of the red pigment. Just what the supposed common step is in this case, and what its relation may be to the common step controlled by the white gene, is not known.

It should be emphasized that the scheme presented in Fig. 1 is tentative and hypothetical. It is to be expected that it will have to be modified as more information becomes available. Throughout the scheme we have indicated genes acting through the intermediation of enzymes. In a sense this is a purely gratuitous assumption, for we have no direct knowledge of the enzyme systems involved. Since, however, we know that in any such system of bio-

logical reactions, enzymes must be concerned in the catalysis of the various steps, and since we are convinced by the accumulating evidence that the specificity of genes is of approximately the same order as that of enzymes, we are strongly biased in favor of the assumption. In this we make no claim to originality, for it has many times been suggested by geneticists that there may be a close relation between genes and enzymes. It is, of course, possible that the immediate products of many genes may be enzymes or their protein components. At the present time, however, the facts at our disposal probably do not justify the elaboration of hypotheses based on this assumption.

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EFFECT OF INDUCED POLYPLOIDY IN PLANTS[1]

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PERHAPS the most effective method of treating the subject of my talk this afternoon would be to give an abstract of the interesting series of papers on polyploidy in this morning's joint symposium, which was organized by Dr. Nebel. Since this is not possible in the time available, I shall restrict my talk to only a small section of the field and tell you about some of the work on polyploidy recently carried on in our laboratories, much of which has been presented by my colleagues in more detailed papers elsewhere at this meeting.

The term ploidy indicates duplication and is used in relation to number of chromosomes. Thus the sex cells which have only one chromosome of a kind may be called monoploid; the body cells which have two chromosomes of a kind are diploid; those with four of a kind tetraploid, and so on to hexaploid, octoploid and higher orders of the polyploid series.

A number of methods for obtaining plants with doubled chromosome number are now available. Heat was successfully used by Randolph and others for this purpose. Recently colchicine has been found to be a relatively simple stimulus to chromosome doubling.[2] Certain other

[1] Read at a symposium presented at a joint session of the American Society of Naturalists with the American Society of Zoologists, the Botanical Society of America, the Genetics Society of America and the American Society of Plant Physiologists, at the meeting of the American Association for the Advancement of Science, Philadelphia, January 1, 1941.

[2] Since we have been frequently asked what suggested colchicine as a means of inducing chromosome doubling, the history of its use in our laboratories may be briefly recounted. The effects of colchicine in arresting nuclear divisions and its use by endocrinologists to determine the stimulation of tissues by hormonal action was brought to our attention by a Journal Club review of a paper which had been given at the Atlantic City meeting of the A_sS by Edgar Allen, G. M. Smith and W. U. Gardner, entitled "Accentuation of the Growth Effect of Theelin on Genital Tissues by Arrest of Mitosis with Colchi-

chemicals will also induce the doubling of chromosomes. Acenaphthene has been recommended, but we have found it a relatively poor reagent for the purpose. Phenyl urethane within a narrow range of concentrations near saturation has been effective in treating seeds of *Datura*. Ultimately a better inducer of tetraploidy may be discovered, but nothing so far tested by us has proven so good as colchicine.

Let us consider some of the most conspicuous effects of doubling chromosome number. That of most interest to laymen is the increased size of flowers. In Fig. 1A on the left is shown a normal diploid ($2n$) flower of the garden Petunia and a tetraploid ($4n$) flower on the right. The increased size of the tetraploid flower is obvious—too obvious in fact to be a true example of the effects of doubling chromosome number. Although the figure may appear to resemble the before-and-after advertisements for vitamin B_1, precautions were taken to secure typical examples. One branch of a Petunia plant was transformed into a tetraploid by treatment with colchicine and

cine." In the discussion which followed we remarked that anything which made chromosomes behave differently ought to be experimented with by botanists. A little later our former assistant, Dr. Eigsti, who at that time was assistant to Dr. Demerec, told us on leaving the Department that he had found colchicine would double chromosome number in roots of several plants with which he had experimented. He suggested we try it with *Datura*. We told him we had planned using it with *Datura* seeds but could not be very hopeful of success, since we had failed to get $4n$ shoots by the use of various agents, including chloralhydrate with which Nemec had induced $4n$ cells in roots as early as 1904. Chromosome numbers appear capable of doubling up rather readily in roots and we have frequently found, for example, that the roots of our monoploids have been partially or wholly diploid. It appears a much more difficult task, however, to induce doubling of chromosomes in the shoots which alone are capable of producing flowers and seeds and thus giving rise to tetraploid races. However, colchicine turned out to be a very effective stimulus for the production of tetraploids by seed treatment. About the same time the Nebels independently succeeded in getting tetraploid plants by the use of colchicine following a suggestion from D. F. Jones, which had been stimulated apparently by the same paper which has already been cited. Earlier papers having to do with the peculiar action of colchicine and other drugs on chromosome behavior have been cited elsewhere and need not be discussed here. It was inevitable that as soon as their significance was realized they would be utilized in genetical engineering.

its 4*n* offspring were planted in the garden alongside of the 2*n* offspring from the diploid control branches. Tetraploids are slower in flowering than diploids, and the photograph represents the first tetraploid flower that opened and a typical 2*n* flower. For some reason this 4*n* flower was abnormally large for its chromosome consti-

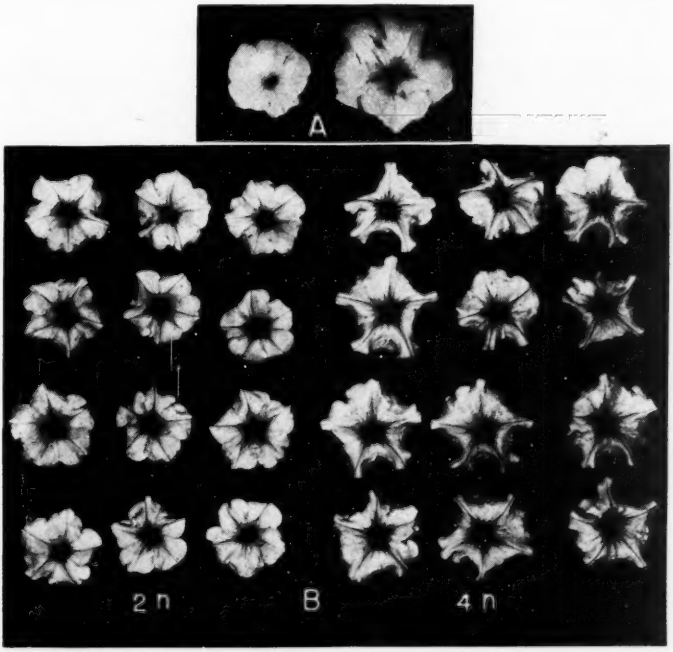


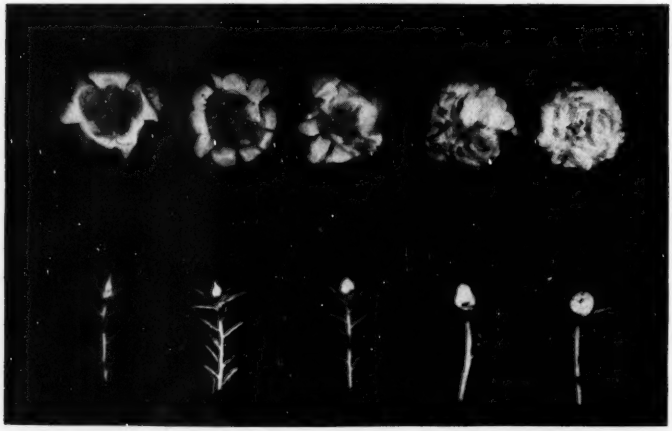
FIG. 1. Flowers of garden Petunia: *A*. On left a normal 2*n* control, on right the first flower from our 4*n* cultures.

B. 12 flowers on left from a single 2*n* plant, the 12 on the right from a single comparable 4*n* plant.

tution. A fairer representation is that in Fig. 1B in which a dozen flowers from a typical 4*n* plant are photographed in contrast to a dozen from a typical 2*n* plant. The 4*n* flowers average larger. This is the general rule. With *Portulaca*, however, (both *P. grandiflora* and *P. parana*) the 2*n* and 4*n* types can not be distinguished by

flower size alone and the Nebels have told us that the same is true of certain strains of Petunia.

In *Portulaca* we have an example of new floral types brought about by tetraploidy. In this species, double flowers (D) are dominant to singles (d). Full doubles (D_2) do not set seeds; semi-doubles (Dd), however, are fully fertile. In diploids there are two types of doubles (D_2 and Dd); in tetraploids there are four. These are



d_4 Dd_3 D_2d_2 D_3d D_4

FIG. 2. Different types of double flowers from a segregating culture of $4n$ *Portulaca grandiflora*.

shown in Fig. 2. The two new double types are D_3d and Dd_3. The homozygous tetraploid (D_4) does not set seed, but the triplex type (D_3d) is partially fertile. If segregation in this case is in accord with random assortment of 4 chromosomes, the plant breeder could guarantee that all the seeds from selfing triplex plants would produce high-grade doubles. If, however, segregation followed random assortment of 8 chromatids there should be not over 0.13 per cent. singles.

Fig. 3 shows pollen of diploids and tetraploids of a number of species. In all cases pollen of the $4n$ is at the right. So far we have found no exception to the rule that

doubling chromosome number increases notably the size of the pollen grains. Pollen size has therefore been a shortcut method of determining tetraploid flowers. Wettstein has reported that after several generations of inbreeding the size of spores in tetraploid capsules of certain species of mosses with which he had experimented had returned to the size characteristic of diploid capsules. No similar reduction in volume of cells with doubled chromosome number is known among flowering plants. In *Datura stramonium* we have two 4*n* lines which have

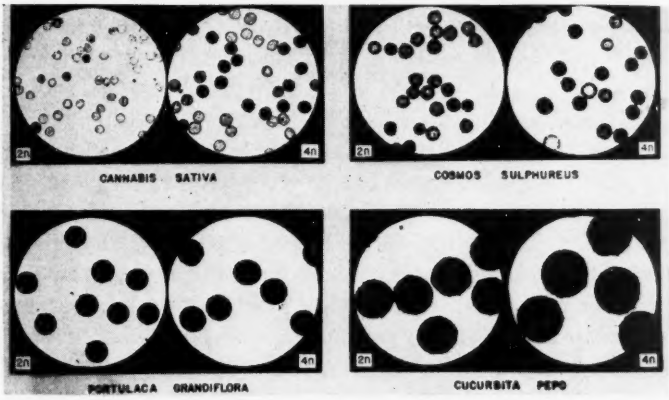


FIG. 3. Pollen of four species. In each case the pollen on the right from a tetraploid is larger than that on the left from a 2*n* control.

been inbred through selfing for 13 and 14 generations, respectively. Measurements show that their pollen grains are the same size as those of 4*n* plants which were the immediate offspring of induced tetraploids.

Fig. 4 shows seeds of different species. In each case the 4*n* seeds on the right are larger than the 2*n* seeds of the same species.

In Fig. 5 are shown capsules with their contents of the balanced polyploid series of *Datura stramonium*. Only the 2*n* capsule contains a full quota of seeds; seeds from a 4*n* capsule are fewer in number and larger in size; only a single seed, seen at the right of the cluster of aborted

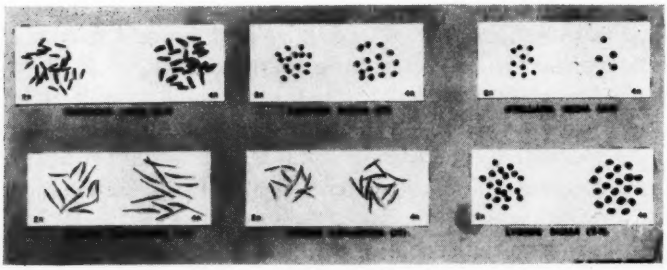


FIG. 4. Seeds of six different species. Upper row: *Rudbeckia hirta, Cannabis sativa, Stellaria media.* Lower row: *Cosmos sulphureus, Bidens leucantha, Lychnis dioica.* In each case tetraploid seeds are larger than diploid controls on left. The figure is rephotographed from an exhibition chart and magnifications should be disregarded.

seeds and ovules, has been obtained from 6*n* capsules, and this one failed to germinate; the 8*n* capsules have never produced any seeds; the odd-balanced 1*n* capsule occasionally has a few seeds, presumably produced by non-reduction; the seeds of the odd-balanced 3*n* capsule are reduced in number and vary in size due to the varying number of chromosomes which they contain. There appears to be a limit to the number of times the chromosomes can be doubled in plants. In *Datura* we have had

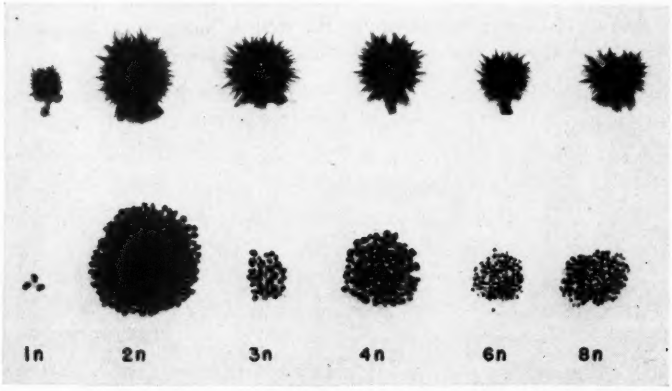


FIG. 5. Capsules from a polyploid series in *Datura stramonium.* Below each is seen the contents of the capsule.

6*n* and 8*n* flowers but have never succeeded in securing 6*n* or 8*n* plants. In *Portulaca parana*, however, we have obtained rather weak 8*n* individuals which were unbalanced by deficiencies for several chromosomes. Species probably differ in respect to number of times their chromosomes may be doubled without serious loss of vigor. The shape of the capsule is determined to a certain extent by the number of seeds it contains. Taking into consideration the fact that the unbalanced 1*n* and 3*n* capsules have relatively few seeds, it will be seen that there is a progressive shortening and thickening of the *Datura* capsules shown in Fig. 5 as one goes from the 1*n* to the 8*n* capsule of our highly inbred line of *Datura*.

An interesting series of shape changes due to chromosome doubling has been found in fruits of the *Cucurbitaceae*. Sinnott, with whom we have been cooperating in his study of factors in growth and differentiation, has discovered that the effect of polyploidy upon the relative proportions of the cells and the frequency of divisions in different planes depends greatly upon what species is affected—in other words, upon the genic constitution of the chromosomes which are doubled. The end results are shown in Fig. 6. In the Spoon Gourd (A) the 4*n* fruit is large in all dimensions though somewhat wider than the 2*n* fruit. In the Club Gourd (B) which has a narrow and greatly elongated 2*n* fruit, the 4*n* fruit is much shortened and thickened. The 2*n* Pear-shaped Squash (C) is longer than wide, while the fruit of the 4*n* is disc-shaped—a difference in shape which Sinnott has found in other 2*n* races to be due to individual genes. The Bottle Gourd (D) has a much constricted neck when 2*n*, but the neck is entirely eliminated when the fruit is 4*n*.

One of the most fruitful sources of new species in nature and of superior varieties of cultivated plants is to be found in multiple diploids. They originate from hybrids which might be called mule plants since their chromosomes like those of the mule are too distantly related to mate and form pairs, and without chromosome pairing

sexual reproduction is impossible. After the chromosomes of such sterile hybrids are doubled each chromosome has a mate from its own species with which to pair and the hybrid becomes a fertile double diploid. Spontaneous doubling of chromosomes to form multiple diploids from sterile species hybrids has been the origin of the best varieties of oats, wheat, tobacco and cotton.

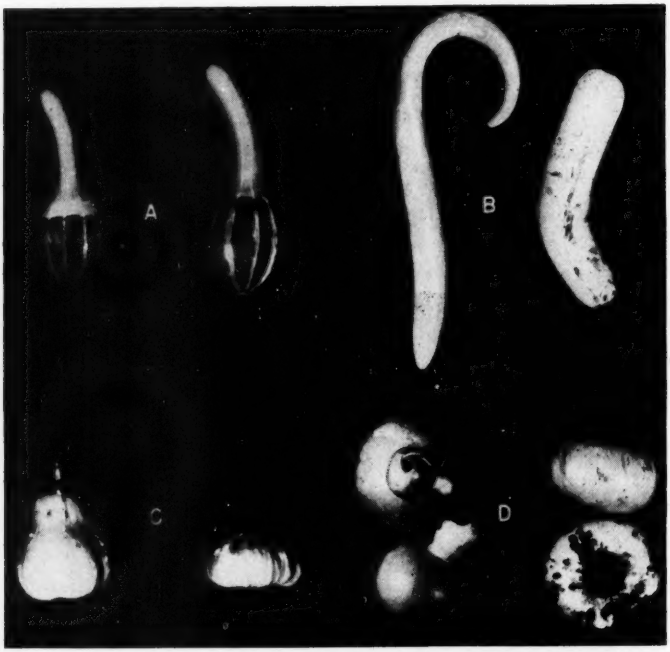


FIG. 6. Diploid and tetraploid fruits of cucurbits. In each case the fruit at right is $4n$ and that at left is $2n$ control.

A. Spoon Gourd. B. Club Gourd. C. Pear-shaped Squash. D. Bottle Gourd.

Methods of producing double, triple and quadruple diploids from sterile hybrids have been discussed in earlier papers.

We have been discussing the transformation of a sterile into a fertile form through doubling chromosome num-

bers when the sterility is due to practically complete incompatibility between the chromosomes of the parents of the sterile hybrid. Sterility may be brought about by a number of different causes and we should not expect to be able always to relieve it by doubling the chromosome number. We have in *Datura* an example of a considerable reduction in the amount of pollen sterility through

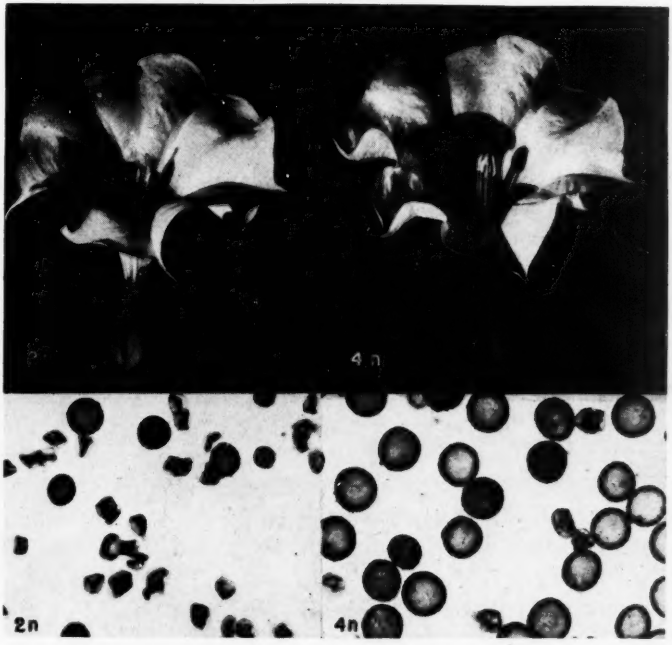


FIG. 7. Hybrid *Datura metel* × *D. meteloides*.
Above, 2*n* and 4*n* flowers. Below, pollen from 2*n* and 4*n* flowers.

doubling chromosome number without the formation of a double diploid. In Fig. 7 is shown on the left a 2*n* flower of the species hybrid *Datura metel* × *D. meteloides*. The cross between these two species is very difficult to make and only a single hybrid plant had been secured from several hundred pollinations when the photograph of Fig. 7 was taken. Most of the chromosomes of the parental spe-

cies have different end arrangements such that at metaphase the hybrid shows two circles of 8, one circle of 4 and two pairs of chromosomes. There are thus only two chromosomes of the two species which are alike in end arrangement. The circles tend to break prematurely, as is often the case in species hybrids, and the chromosomes to align themselves on the spindle as unequal pairs and singles. In consequence, chromosome deficiencies are brought into the developing pollen mother cells with a resulting high percentage ($\pm$ 85 per cent.) of aborted pollen. On the right in Fig. 7 is shown a $4n$ flower from a branch of the hybrid in which the chromosomes had been doubled by spraying the plant with colchicine emulsion. The plant had proven rather resistant to treatment and it was only after about a year's intermittent attempts that we succeeded in getting a branch all the flowers of which were tetraploid. The tetraploid flower shown in the photograph was only slightly larger than the $2n$ control on the left. The corolla, however, was of considerably thicker texture. It will be noticed that the filaments of the stamens are thicker and the style much shorter than in the $2n$ flower. Dr. Bergner has found that the chromosomes of the $4n$ hybrid tend to assort together with an increased number of bivalents. It is for this reason probably that pollen abortion in the $4n$ flower has been reduced to only about 25 per cent. The lower part of Fig. 7 shows pollen of the $4n$ hybrid on the right in comparison with that of the highly sterile $2n$ hybrid on the left.

With *Anagallis* we have rendered a sterile hybrid fertile by treatment with colchicine without being sure of the cause of the initial sterility. Marsden-Jones and Weiss reported sterile hybrids between the closely related subspecies *Anagallis arvensis phoenicia* × *A. arvensis foemina* except when the former was the salmon-flowered variety, in which case hybrids were fertile. We suggested to Professor Weiss that he test the effects upon the sterility of doubling the chromosome number. He

sent us seeds to produce the hybrids and asked us to make the tests. The species proved to be very susceptible to treatment, a single spraying with an emulsion containing 0.4 per cent. colchicine being sufficient to induce fertility. The leaves of the treated branch were characteristically thickened and the growth in this region checked. By cutting off the vigorous shoots which grew from the untreated buds below, growth was induced from the treated region. The chromosomes have not yet been counted, but

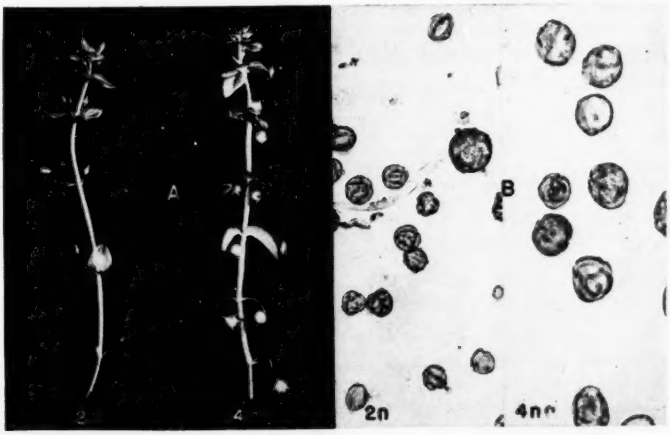


FIG. 8. Hybrid *Anagallis arvensis* var. *phoenicea* × *A.a. var. foemina*.
A. Left, sterile hybrid; right, branch rendered fertile by colchicine.
B. Pollen of *Anagallis* hybrid: on left from sterile hybrid; on right from fertile treated branch.

judging from the appearance of the leaves on some of these branches their chromosomes have been doubled. Good pollen from flowers on such branches is shown on the right in Fig. 8B in comparison with aborted pollen of the untreated sterile hybrid. Flowers with good pollen produced capsules with good seeds (Fig. 8A, right) while the flowers of the controls (Fig. 8A, left) fell off without setting fruit. It will be seen that even the pollen test is not necessary to determine that the sterile hybrid has been rendered fertile by colchicine treatment.

It is often assumed that treatment with colchicine or other polyploidizing agents, if effective, will induce an exact doubling of each chromosome so that a balanced $4n$ condition, for example, will result throughout the part of the plant affected. This is very far from what actually happens. The large majority of affected sectors from heavily treated *Datura* have been unbalanced by the loss of one or more chromosomes or have consisted of a mixture of cells with different chromosome numbers.

Fig. 9 shows in diagram some of the deficient types

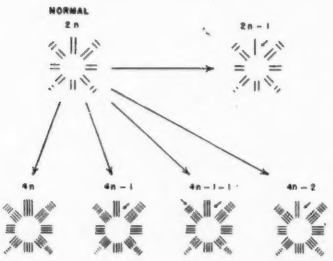


FIG. 9. Diagram showing chromosome deficiencies as well as chromosome duplication in *Datura* induced by colchicine treatment.

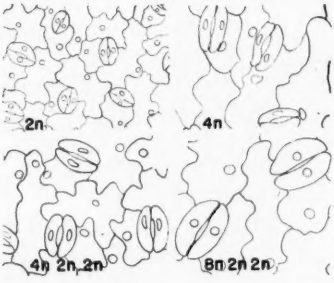


FIG. 10. Guard cells from epidermis of $2n$ and $4n$ leaves of *Datura* and from leaves of periclinal chimeras with $4n$ and $8n$ epidermis.

identified by Dr. Bergner. Chromosomes may be eliminated either before doubling to form $2n-1$ sectors or after doubling to form $4n-1$ sectors. As many as 6 chromosomes have been found missing from the 48 of a normal $4n$ condition. Frequently the doubling occurs after elimination to form $4n-2$ types in which two chromosomes are missing from one of the twelve sets. The frequent loss of chromosomes accompanying induction of tetraploidy has been an inconvenience in our breeding program. Thus the flower of the species hybrid which is represented in Fig. 7 as a tetraploid was later shown to have 47 chromosomes and to be therefore a $4n-1$ type rather than a balanced tetraploid.

A further departure from a pure polyploid condition following colchicine treatment is seen in periclinal chimeras in which at least one of the three germ layers differs from the others in chromosome number. Fig. 11 shows some of the periclinal types in *Datura* which have been determined by Miss Satina. The chromosome numbers have been determined from dividing nuclei, but the size of the cells and especially of their nuclei in sectioned material form good criteria of the polyploid condition. The size of guard cells of the epidermis (Fig. 10) if taken

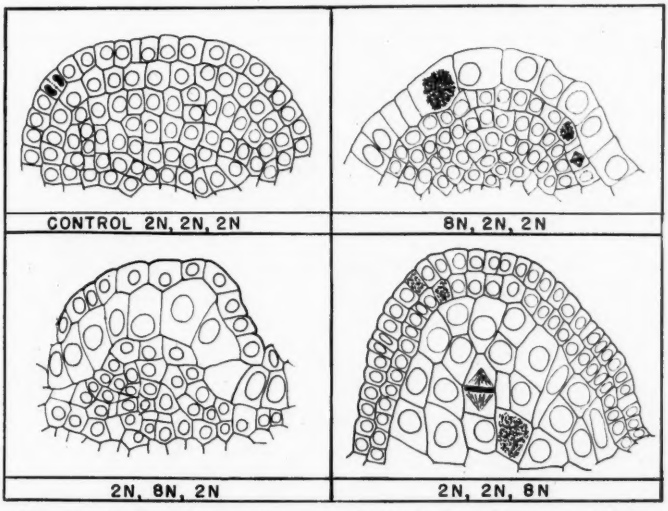


FIG. 11. Longitudinal sections through shoot apex of 2*n* control *Datura* and of three different types of periclinal chimeras in each of which one of the three germ layers is octoploid.

from comparable leaves gives an indication of the chromosome number of the outer germ layer. Egg cells and pollen are formed by the second layer and in consequence the chromosome number of this layer can be approximated by inspection of the size of pollen grains or accurately determined by chromosome counts in pollen-mother-cells. For determination of the third or innermost layer there is no short-cut method and one must depend upon sectioned material. Field-grown plants from

colchicine-treated seeds were roughly determined by Mr. Avery and myself as to chromosome constitution from their appearance. Later the chromosome constitution of their three germ layers was determined by Miss Satina. It was rarely the case that we did not recognize duplication of chromosomes in any one of the germ layers by differences in the appearance of the plants. Fig. 12 shows the effect on capsule shape of tetraploidy in each germ layer. Tetraploidy in the innermost or third layer had most influence in depressing the capsule.

Periclinal chimeras have enabled us to label the different germ layers and thereby to determine the contribution which each makes to the developing organs. They help in analyzing the relationships between organs. Thus, the evidence from periclinal chimeras indicates that the petal and leaf develop in early stages chiefly from the second germ layer and may be considered to be phylogenetically related. In the developing stamen, however, the third or innermost layer contributes a major portion of the young organ. The stamen therefore can not be closely homologized with petal and leaf, as many have believed. Periclinal chimeras are readily obtained and appear to furnish a new tool for solution of problems of development and phylogeny.

A study of the pistil by means of periclinal chimeras has shown that the central portion of the style through which the pollen tubes grow is formed like the epidermis from the outermost germ layer. This fact explains certain peculiarities in the setting of capsules on different periclinal chimeras. Buchholz has shown that when a tetraploid female ($4n$) is pollinated by a $2n$ male the $1n$ pollen tubes grow well in the $4n$ conducting tissue of the style. In the reciprocal cross, however, when the $4n$ is the male parent, the $2n$ pollen tubes burst in the conducting tissue of the $2n$ styles. In Fig. 13 are shown two periclinal chimeras of *Datura*. The one on the left is $4n$, $2n$, $2n$ labeling the three germ layers in order from the outside in. The conducting tissue of its style comes from the

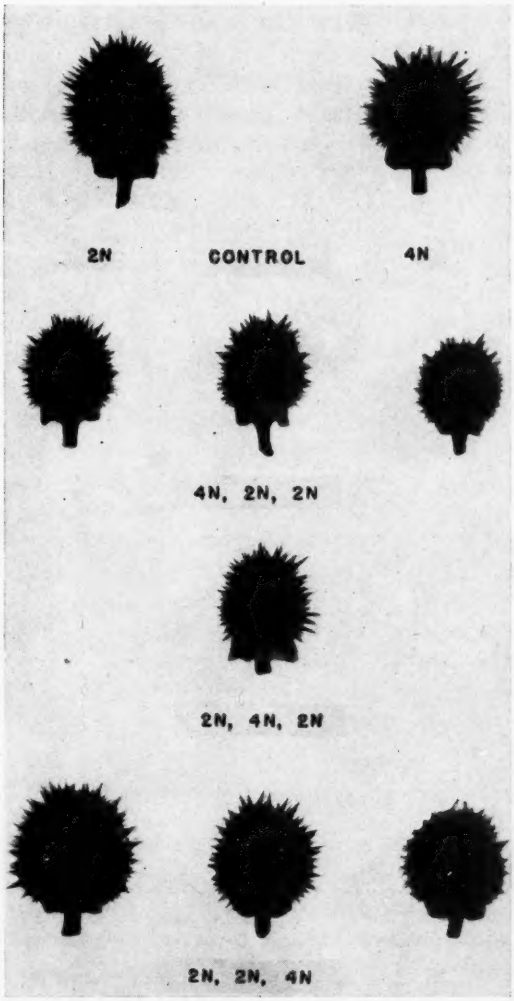


FIG. 12. Capsules of periclinal chimeras of *D. stramonium:* First row, capsules of 2*n* and 4*n* controls; second row, three capsules in which the first germ layer is 4*n;* third row, one capsule in which the second germ layer is 4*n;* fourth row, three capsules in which the third germ layer is 4*n*.

first layer and is $4n$, and therefore is able to allow the growth of the $1n$ pollen tubes from the second layer which is $2n$. It is for this reason that this periclinal chimera has set several capsules by selfing. The plant on the right is $2n$, $4n$, $2n$. The conducting tissue of its style is $2n$, while the $4n$ second layer gives rise to $2n$ pollen tubes which

FIG. 13. Two periclinal chimeras of *Datura*. Plant on left is able to set capsules since its $1n$ pollen tubes derived from the $2n$ second germ layer is able to grow through the $4n$ conducting stylar tissue which is derived from the first germ layer. Plant on right does not set capsules because $2n$ pollen tubes from $4n$ second germ layer generally burst in $2n$ stylar tissue.

burst in the $2n$ conducting stylar tissue. For this reason no selfed capsules were formed on this plant.

Buchholz has earlier shown that pollen-tube behavior is a block to crossability between certain species of *Datura*. His recent study of selfs and inter se pollinations among tetraploids of ten species of the genus indi-

cates that pollen-tube growth is generally deleteriously affected by doubling chromosome number. In *Datura,* therefore, we find no support for the belief that increased chromosome number facilitates crossability. The evidence seems to indicate that tetraploidy tends to offer a block to crossability between species. To the extent to which this is the case tetraploidy can be considered to have evolutionary significance in bringing about biological isolation of types evolved through chromosome doubling.

The finding by Hagerup of a 4*n* hermaphroditic species, *Empetrum hermaphroditum,* which was believed to have originated from the related dioecious species *E. nigrum,* gave presumption to the belief that doubling the chromosome number of a dioecious species would result in an hermaphroditic form. Furthermore it has been stated by a number of *Drosophila* investigators that tetraploidy could have played no rôle in the evolution of dioecious species on account of difficulties that would arise in the chromosome mechanism of sex determination. It was for these reasons that we started in cooperation with Warmke tests of the effects of induced tetraploidy in a series of dioecious species of plants. In *Melandrium* we were able to show that doubling chromosome numbers ultimately leads to a balanced tetraploid population with approximately equal numbers of male (XXXY) and female (XXXX) individuals. It would appear therefore that doubling chromosome number does not necessarily lead to hermaphroditism and that in one at least of the species which have been carefully studied there is nothing in the chromosome mechanism that would prevent tetraploidy from playing a rôle in evolution of a dioecious species in nature as it has played a rôle in the evolution of a constant dioecious race in our laboratory cultures. As a matter of fact there are species of *Salix* which have twice as many chromosomes as other species in the genus and the same is true of the genus *Valisneria.* Both of these plants are dioecious.

By changing the proportion of X and Y chromosomes in *Melandrium,* Warmke has been able to control sex expression and to obtain functional hermaphrodites in 4*n* races of this normally dioecious species. By crossing 4*n* females with 2*n* males, triploids were obtained and from the latter offspring were secured with a wide range of chromosome numbers. Among the latter were diploid types with one Y and two X chromosomes which were hermaphroditic. They have been selfed and have given offspring, some of which have the same sex chromosome constitution as their parent and are again hermaphrodites. We plan selfing them for several generations in order to purify the strain before isolating male and female individuals characteristic of *Melandrium.* By having regard to the change of balance of the sex chromosomes we have been able to get offspring from a dioecious form and thus secure the advantages to a breeding program in dealing with hermaphroditic forms, the genetic constitution of which can be readily determined by selfing.

Now that we have means of readily doubling chromosome number, one of the great needs in our genetic program is a method whereby we can reduce the number by half. If we could produce monoploid plants at will which we could later change to diploids by colchicine treatment we would have a means of securing in two jumps pure lines from highly heterozygous material. That the problem is probably soluble is indicated by the fact that the plant has solved it. In *Datura,* for example, we have recorded the spontaneous occurrence of over 225 monoploid plants which have presumably come from 1*n* egg cells which had grown into embryos without having been fertilized. For some time we have attempted to induce by artificial stimuli the development of such parthenogenetic eggs but so far without success. By the use of auxins, however, it has been possible to induce the capsules to stay on the plant and to enlarge without pollination, but no viable embryos were formed. This past summer the problem was attacked anew by Drs. van Overbeek and

Conklin. They failed to induce true parthenogenesis leading to $1n$ seeds. They did however induce in *Datura* by the use of auxins injected into the ovary the production of pseudoembryos in the place where embryos normally develop. They are formed by ingrowths from $2n$ maternal cells just outside of the embryo sac. The pseudoembryo is of considerable size. If it could be induced to differentiate into a true embryo we would have a method of reproducing monoploids and triploids by apogamous seeds. Of considerable economic importance also would be such a method which would enable the plant breeder to have the advantages of seed reproduction when working with sterile forms and to avoid segregations when breeding heterozygous material.

Heretofore, conclusions regarding the effects of polyploidy have had to be drawn for the most part from species in nature which differ in chromosome numbers or from occasional spontaneous occurrences of tetraploids in races which were far from homozygous. It is now possible to induce polyploidy in controlled material and from its study to establish general laws regarding the effects, physiological as well as morphological, which are brought about by duplication of chromosomes.

I have given an inadequate account of some of the recent work which has been carried on in a single laboratory. It should not be forgotten that many others have made contributions in this field and that our knowledge of polyploidy and its effects is being rapidly widened. The realization that vital processes of the plant are subject to control by chemical stimuli in the hands of the experimenter gives assurance of further progress.

AN ANALYSIS OF FEATHER COLOR PATTERN PRODUCED BY GRAFTING MELANOPHORES DURING EMBRYONIC DEVELOPMENT[1]

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INTRODUCTION

BY grafting melanophores from one embryo to another of a genetically different breed of fowl or species of bird a means is furnished for analyzing the expression of the different genetic constitutions of the combined donor and host tissues in the characterization of feather color pattern. This combination has proved to be a method *par excellence* for getting at the rôle that each component part plays in the control of feather coloration and color patterns. The inherent capacity of the melanophore to act independently and to react to extrinsic factors of the host in controlling pigmentation may be roughly determined. This paper deals primarily with the nature of this control, especially as it occurs in the formation of barred patterns.

In this study the melanophores of various breeds or species of birds are introduced into the feather germs of white and pigmented host fowls by transplanting potential melanophores (skin ectoderm and underlying neural crest cells) from a donor embryo of about 70 hours or an equivalent age into the right wing bud of a host of the same age. This results in the formation of an area of donor-colored down feathers on the wing and adjacent regions in the majority of cases. The down is replaced by juvenile contour feathers having the shape, rate of growth and distribution in tracts characteristic of corre-

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sponding regions of host control chicks but invariably the color or color pattern of the donor breed or species.

From several lines of evidence (see Willier and Rawles, 1940; also Dorris, 1939, Eastlick, 1939, Rawles, 1939 and Ris, 1941) it has been proved that melanophores migrate out from the implant into the host epidermis and feather germs and produce the area of donor-colored feathers. Donor melanophores from pigmented birds deposit melanin granules, which are breed or species specific as to size, shape and color, in the epidermal cells of the shaft, barbs and barbules of the host feathers. Melanophores from white breeds (4 examined) likewise enter the host feather germ but deposit few or no melanin granules with the result that the host feather is white. It has been shown by Hamilton (1940a) by means of *in vitro* studies that these melanophores, although capable of synthesizing melanin, have a much lower viability and higher sensitivity to adverse environmental conditions than melanophores from birds having pigmented plumage. Apparently, as the melanophore grows some lethal factor brings about the onset of changes which leads to its premature death.

Genotypic Control

Our results have shown that the coloration or color pattern produced in the host feather is in accord with the genotypic composition of the donor melanophore. Melanophores from an embryo of a bird having a uniform one-colored feather (*e.g.,* Black or Buff Minorca, White Silkie, etc.) bring about the same uniform coloration in the host feather. Those from a donor species having a bi- or multi-colored pattern (*e.g.,* Barred Plymouth Rock, Brown Leghorn, guinea fowl, N.H. Red, robin, etc.) produce the same kinds of color pattern in the host feathers no matter what breed of host is used.

Barred melanophores produce a barred pattern in host contour feathers of non-barred breeds such as N.H. Red, White Leghorn and Black Minorca, and of a Mongolian pheasant (Rawles, unpublished results). Two types of

barring, one darker than the other, occur. In the darker pattern the black bars are wider and darker than in the lighter one. These differences are similar to sex-linked differences in plumage found in donor control chicks of the same age where the female plumage is darker than the male. The comparison indicates that a melanophore from a female donor which has only one gene for barring produces a darker-colored host feather than one from a male which has two genes.

The same principle of genotypic control of pattern holds when melanophores from a hybrid cross (Barred Rock ♀ × R.I. Red ♂) are used. Melanophores from ♂ and ♀ embryos (sex ascertained after donor is hatched) produce respectively barred and non-barred contour feathers in a White Leghorn host. The sex of the host has no effect on the result. Here only the melanophores from the male embryo which contain a gene for barring produce a barred pattern in the host feather.

In a similar way melanophores from the guinea produce plumage patterns—(*i.e.*, stippled, barred and spotted) in White Leghorn feathers like those found in corresponding feathers of control guineas.

Mode of Action of Barred Melanophores

The results show that only melanophores containing genes for barring can produce a barred pattern in host feathers. They do so in all the tested breeds of hosts having non-barred plumage such as the N.H. Red, Black Minorca and White Leghorn. Furthermore, melanophores from non-barred breeds are unable to produce a barred pattern in Barred Rock hosts, showing clearly that any possible inherent barring rhythm in the Barred Rock host either in the feather germ or the body is not effective when combined with non-barred melanophores. In other words, the epidermal substratum of the feather germ alone can not determine the barring pattern. This points to the interpretation that the capacity to produce barring depends upon the melanophore.

That the barred melanophore reacts to extrinsic factors in the host is brought out very nicely by an examination of the variation in barred patterns formed in White Leghorn host feathers. In certain flight feathers where the growth rate is rapid the black bar is very wide and often not sharply defined, *i.e.,* the black shades off to white gradually, giving a brownish coloration. In some cases the flight feather is almost wholly black, only the shaft showing traces of alternating bands of white. In a slow-growing one such as the breast feather the amount of white is much greater and the alternate bands of white and black are very distinct, *i.e.,* the line of demarkation between them is straight and sharp. In feathers having an intermediate rate of growth such as the wing coverts a still different type of barring is evident. Here the white bands are narrower and the black bands wider than in the breast feather. Thus we see that though the melanophores all came from the same region (head) of the donor embryo the pattern produced varies with the time of emergence and the position of the feather on the body of the host.

In effect the same kind of variations in host feathers is found when melanophores from the F_1 hybrid male embryo are grafted to a White Leghorn host. These experiments are of special significance, since in two cases the feathers of the donor chick can be compared with the pigmented patterns induced in the host feathers. When this is done it is found that the first few primary flight feathers resemble each other very closely. In the case of the axillary and most of the secondaries, however, the host feathers tend to be somewhat uniformly black with a trace of a white bar on the shaft and occasionally show a single white bar across the tip of the vane. In the donor control these same feathers exhibit a barred pattern in which the white bars are somewhat irregular and somewhat indistinct (the barring in the tips of the vanes may be much more distinct and straight). In secondaries 12 and 13 the barring in both donor and host is quite distinct and similar but not identical.

Still more striking and extreme variations in color pattern are produced by guinea melanophores in White Leghorn host feathers. For example, the secondary flight feathers which emerge first are gray with tan-brown tips and the outer vane margins are mottled brownish-gray. Later emerging feathers show irregularly shaped, cream-white bars on a gray background. In the last ones to emerge the Whitish bars begin to break up into irregularly formed areas or "spots." Whitish bars likewise form in the breast feathers and in certain of the upper wing coverts. Such variations in pattern are nearly identical with those found in corresponding feathers in guinea fowl controls. Thus we see that the pattern varies greatly with the time of emergence (rate of growth) and the position of the feather on the wing and breast. It ranges from one which has no resemblance whatsoever to a barred pattern to one which is typically barred.

It is apparent therefore that the individual feather germ alters greatly the type of barred pattern produced. Each feather germ produces its own particular type of pattern. It is also noticed that the greatest variations occur between different feather tracts, *i.e.*, the patterns produced in the primary flight feathers resemble each other more closely than the patterns of feathers in the breast tract and *vice versa.*

From the foregoing analysis it is clear that the kind of barred pattern produced is under the control of the individual feather follicles of the host. This leads us next to inquire whether the seat of the control of the rhythmic alternations of dark and light bands is in the feather germ. Several lines of evidence indicate that functional rhythm is a property of the feather germ. By grafting pieces of skin shortly after hatching from one breed of fowl to another Danforth and Foster (1929) have shown that (1) Barred Plymouth Rock skin grown on a variety of non-barred hosts produces typically barred feathers indistinguishable from controls, (2) skin grafts from non-barred donors grown on Barred Plymouth Rock hosts

produce feathers like the donor in color and pattern, and (3) white birds known to carry the barring factor do not produce barring in feathers of a Black Minorca skin graft. From these observations the investigators concluded "that barring of the Plymouth Rock type is purely a function of follicular activity" (p. 457). Later Montalenti (1934) arrived at a similar conclusion from evidence of a different sort. By comparing split preparations of different regenerating feathers plucked at the same time he found that the synchronism of phase (black or white), although somewhat perfect in follicles of the same regions, was often imperfect or entirely discordant in others. Marks recorded in regenerating feathers as a result of thyroxin injections began in the same phase in the majority of feathers, but in others a discordance of phase was seen. From these observations Montalenti drew the conclusion that "barring is due to a genetic factor, working directly, and to a certain extent independently in the single follicle, and not by means of some endocrine intermediary" (p. 293).

The question now arises as to the rôle that the physiological rhythm of the follicle plays in the formation of barred pattern of the host feather. If the rhythm of the follicle were alone responsible for barring, we might expect it to occur with variations in pattern due to differences in growth rate when Black Minorca or N.H. Red melanophores are grafted into a Barred Rock host. Such is not the case, as we have seen. Consequently, a more logical explanation in keeping with the evidence is that the barred melanophore is an independent system reacting in its own specific way to extrinsic rhythmic factors and growth rate in the epidermal substratum. This would mean, however, that in all fowl hosts having non-barred plumage there is an inherent functional rhythm. This does not seem improbable in view of the fact that our domestic breeds of fowl have a common origin and barred pattern is fairly common among birds in general. The validity of this hypothesis may be tested by grafting

barred melanophores to birds having a physiological rhythm of a different order from that occurring in the common fowl.

Accepting this explanation it follows that the barred melanophore is so adjusted that it responds to fluctuating differences in physiological activity in the follicle, the response varying in accordance with differences in the growth rate of individual follicles. What is the nature of the mechanism of response? The work of Lillie and his collaborators (Juhn and Gustavson, 1930, and Lillie and Juhn, 1932) furnishes a clue to the nature of the physiological mechanisms operating. They have shown from an analysis of pigmentation marks (transverse bars, marginal and axial patterns, etc.) induced in regenerating feathers by injecting hormones that a definite relation exists between threshold of action to hormones and growth rate of the feather. On the basis of such a relationship, Montalenti (1934) attempted to explain the manner of formation of barred patterns in Barred Plymouth Rock feathers. He showed in general that more rapidly growing feathers or parts have a short white phase and a black phase of longer duration, indicating a higher threshold of action to the white determining factor. Conversely, in slowly growing feathers the white phase is long and the black phase shorter, hence a lower threshold of action.

Applying this principle to the action of the barred melanophore in a foreign host feather we note some significant results. In a rapidly growing host feather such as the flight feathers the amount of white is greatly reduced, thus forming patterns resembling but not identical with Barred Rock control flight feathers. Measurements of the rate of growth in length of the fourth and fifth primaries show, however, that these feathers are growing much more rapidly in the White Leghorn host than in the Barred Rock controls (see Fig. 1, Willier and Rawles, 1940). The barred pattern formed in the White Leghorn feather is consequently different from that produced in a control Barred Rock feather. In some of the primaries

it is almost uniformly black, only traces of white being found. When the growth rate is lower as in the breast feather the white phase is longer and the black phase is shorter.

In the N.H. Red host, where the rate of growth of many of the flight feathers is somewhat intermediate between that characteristic of corresponding feathers of a White Leghorn and Barred Rock, still different barred patterns are produced. By comparing the seventh and eighth secondaries in these birds it is found that in the White Leghorn feather the alternating dark and light bands are scarcely visible at all except on the shaft, in the N.H. Red feather they are only distinct in the tip of the vane and shaft, whereas in the Barred Rock control they are more or less evident throughout the vane but much more pronounced at its apex and base. In general, the pattern produced in the N.H. Red feather resembles more closely that of the Barred Rock control than it does that formed in the White Leghorn feather. Apparently the rate of growth of the feather germ of the N.H. Red approximates more closely that of the Barred Rock than it does that of the White Leghorn.[3]

It is apparent, therefore, that the barred melanophore responds to inherent rhythmic activity of the feather germ, the pattern actually formed being dependent upon the growth rate of the individual feather germ. The barred melanophore is so constituted that it can respond to such differences. It apparently functions continuously (deposits pigment granules) when the threshold to the white determining factor remains at a very high level, giving an almost completely black feather. Even during the period when the white factor is operative in other feathers, the threshold necessary for the formation of black pigment is still maintained in flight feathers. In a

[3] It should be pointed out here that in the Barred Plymouth Rock the barred pattern in homologous feathers varies considerably from individual to individual. According to Martin (1929) such differences in quality of barring are to be correlated with variations in the rate of feather growth found in different chicks of this breed.

feather of intermediate growth rate the melanophore responds differentially to the fluctuating conditions giving white and black bars of nearly equal width. An examination of a split regenerating feather germ of a Barred Plymouth Rock shows that although melanophores are present in the white band and are depositing some pigment granules in the feather parts they are much less numerous than in the black band. The difference in the two bands appears to be merely quantitative so far as the melanophores are concerned. It is conceivable therefore that the conditions in the epidermal substratum during the white phase are unfavorable, whereas in the black phase they are favorable for the multiplication of melanophores. A Black Minorca melanophore, on the other hand, is so constituted that it does not respond to these inherent physiological differences in the feather germ. Its action is continuous in feather germs having the inherent rhythm (Barred Rock). Furthermore, differences in growth rate have little or no effect on the deposition of pigment in the host feather. It is evident then that the response of the Black Minorca melanophore is such that melanin granules are deposited continuously through both phases of the rhythmic cycle.

General Interpretation

How donor melanophores of one genetic composition combined with a host feather germ of another genetic composition appear to cooperate in forming pigment patterns is represented schematically in Fig. 1. The melanophore has a specific innate reaction capacity, responding to distinctive physiological conditions within the individual feather germ. Growth rate of the feather germ or regional differences within it are effective factors in controlling the number and direction of differentiation[4] of

[4] In the case of the robin and N. H. Red it was argued elsewhere (Willier and Rawles, 1940, pp. 192 and 193) that the undifferentiated melanoblast has the ability to differentiate into either red or black melanophores, the direction being probably determined by regional differences in physiological action within the developing feather germ.

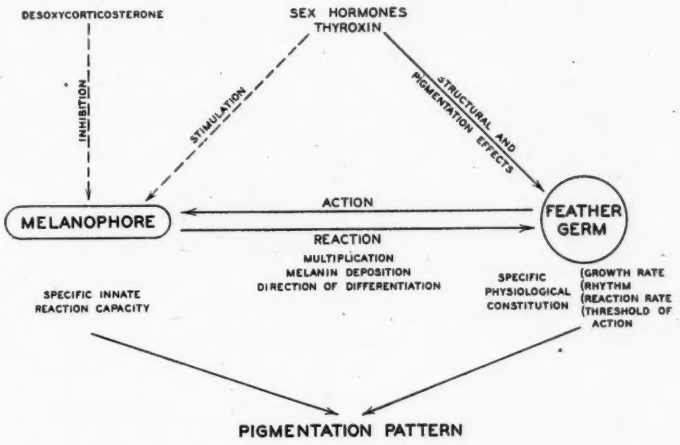

FIG. 1. Diagram illustrating the interaction of melanophore, feather germ and hormones in the production of pigmentation pattern.

melanophores and their deposition of pigment granules into the epidermal cells. In other words, the feather germ furnishes certain physiological conditions which act upon the melanophore. It responds in a specific manner, the kind of response depending upon its genotypic constitution.

That the melanophore may also respond directly to the action of hormones is shown by the recent investigations of Hamilton (1940 a and b). In skin ectoderm explants of the N.H. Red embryo he finds that sex hormones, both oestrone and testosterone, bring about the differentiation of red melanophores (typically only infrequently present in control cultures). Also that desoxycorticosterone, one of the adrenal hormones, will inhibit completely the differentiation of melanophores or bring about a degeneration of those already differentiated. The capacity of the melanophore to respond to hormones leads us to inquire into the mechanisms whereby pigmentation patterns are altered by the presence of these substances in the blood stream. That the hormone acts through the intermediary of the feather germ seems probable, since according to the

work of Lillie and Juhn on the Brown Leghorn the threshold of action to injected hormones varies along the axes of the developing feather germ.

This interpretation serves to make a little more clear cut the nature and interrelationship of the controlling physiological mechanisms concerned in the development of the coloration and color pattern in the feather. The relative extent to which each component part of the complex system influences the pattern of the feather needs to be more fully determined.

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THE HORMONE CONTROL OF PLANT DEVELOPMENT[1]

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THERE are three principal types of hormone so far known in plants: (*a*) hormones controlling flowering, (*b*) the substances belonging to the vitamin B group which are necessary for root elongation, (*c*) the auxins, a group of organic acids which exert their action on many phases of growth and development. One or two others, such as the wound-hormone, or traumatic acid, might be mentioned, but I shall not discuss them here.

About the first little is known; we have only indirect evidence that they exist at all. This evidence is mainly derived from experiments on photoperiodism, that is, the induction (or the prevention) of flowering by control of day length. In the case of "long-day" plants, flower-buds appear after a certain number of exposures to days of 16 hours or more. Now it is only necessary to expose a small part of the plant, in some instances only a single leaf, in order to cause flower formation in a great number of buds. This shows that the local influence of the long photoperiod on the leaf is transmissible to other parts, and suggests a hormonal relationship.

One or more of the group comprising vitamins B_1, B_6 and nicotinic acid are essential for the growth of roots. Different plants have different requirements in this respect. The control, however, is not of a very immediate kind. When root-tips are cut off and grown in culture, and the tip of these culture roots again cut off and transferred to a fresh culture medium, it is found after one or two such transfers that growth slows down and may stop unless the required vitamin is added. But in the first

[1] Read at a symposium presented at a joint session of the American Society of Naturalists with the American Society of Zoologists, the Botanical Society of America, the Genetics Society of America and the American Society of Plant Physiologists, at the meeting of the American Association for the Advancement of Science, Philadelphia, January 1, 1941.

culture from the plant, growth usually goes on at the same rate whether the vitamin is added or not, so that there is evidently a moderate excess present in the root, *i.e.*, the supply is comfortably ahead of the demand. Similarly, the local application of vitamin B, to roots still attached to the plant, does not produce any local acceleration of growth. This is doubtless partly because the amounts required are extremely small (of the order of 0.01 mg per liter of solution) and can thus be readily stored. Probably also the vitamin is not rapidly destroyed, so the excess can accumulate. The situation contrasts notably with the control of shoot growth by auxin, which is much more immediate. Thus the local application of auxin to almost any growing shoot causes a rapid local acceleration of growth, that is, growth is directly and immediately dependent on the available auxin. The most striking example of this is in the tropisms. When a plant is placed horizontally, there is a small diversion of auxin towards the lower side; in the oat and lupine seedlings that have been studied quantitatively this amounts only to a distribution between the upper and lower sides of about 35:65 instead of 50:50. Nevertheless, this small change in auxin distribution causes, within a few minutes, a growth acceleration on the lower side which appears as the well-known geotropic curvature. The influence of light falling from one side is similar, but complicated by the fact that there is not only a difference in supply but also a difference in response. By this I mean that on a given tissue a given amount of applied auxin produces less growth in light than in dark. This is of course the mechanism of the extreme elongation of etiolated plants, and it means that light in some way alters the *sensitivity* of the plant tissue. The reasons for this are still not clear, so I need not discuss them now.

It is an important generalization, then, that variations in shoot growth are directly and immediately referable to variations either in the rate of auxin supply or in the responsiveness to auxin.

Variations in supply may have internal or external causes, and we have one clear-cut example of each—one genetical and one nutritional. In corn, the dwarf variety *nana* (differing from the normal by a single gene) has been shown by van Overbeek to have a rate of auxin production not greatly below normal, but a considerably increased rate of destruction of the auxin formed. The extra rapid destruction accounts qualitatively and perhaps quantitatively for the decreased growth.

In tomato plants grown in nutrient solutions, a deficiency of zinc causes great decrease in growth, and Skoog has shown that before the growth rate changes a marked decrease in auxin content is detectable. The zinc deficiency can in fact be partly compensated for by supplying auxin. Evidently here also the auxin relations determine the growth rate. Nitrogen deficiency also greatly reduces growth and, in parallel, reduces auxin content, but unfortunately nitrogen lack introduces many changes, so that the causal relationship is less certain. However, the fact that low nitrogen is associated with reduced auxin and also with reduced vegetative growth brings to mind another fact, namely, that reduced growth is often associated with tendency to flowering, and suggests that flowering is in some way opposed by auxin supply. Indeed, in *Circaea* it has been shown by Dostál that application of synthetic auxin to the stem causes flower-buds to revert to vegetative shoot-buds. Perhaps the old association of flowering with high carbon: nitrogen ratios may receive its explanation along these lines.

To come back to the morphological results of interfering with the auxin supply, it seems clear that both the element zinc (perhaps also nitrogen) and the dwarfing gene control directly the enzyme systems concerned with auxin.

Variations in response are equally important. The response to auxin differs for different organs and different tissues within the same plant. This is perhaps the most important factor in the control of morphogenesis by

auxin. Specifically, while the elongation of shoots is in general promoted through cell enlargement, under certain conditions auxin may stimulate the cambium to divide and thus produce extra xylem. Also it may induce the cells of the cortical parenchyma, the cambium or the pericycle to undergo rapid division and form root initials. This procedure is, of course, universally used now in the commercial rooting of cuttings. Synthetic auxins, mainly derivatives of indole or naphthalene, are widely sold for the purpose. On the other hand, once the root initial is formed the influence of auxin changes sign, and the elongation of the root is inhibited by auxin. Lastly, the development of buds, especially lateral buds, is powerfully inhibited by auxin. It is the removal of this inhibition, exerted by the growing apical bud through its auxin production, which allows lateral bud development after a shoot has been pruned. An interesting consequence of this is that plants in which the terminal bud is defective in supplying auxin should show greater development of laterals; in other words, dwarf plants become bushy. This is commonly observed both in woody and herbaceous material. For example, dwarf varieties of corn produce many tillers. In two species of aster which differ widely in the degree of their lateral bud development, the plant with the heavier branching was shown by Delisle to have the lower auxin production, while the interspecific hybrid, intermediate in the degree of branching, was also intermediate in its ability to form auxin.

Much has been written about the paradox that auxin, which in general promotes the growth of shoots, should inhibit the development of buds. The effects exerted by the auxins, of course, are multifarious. Now in animals hormones usually perform specific functions and where one gland (like the pituitary) has numerous effects, this is commonly traced to the secretion of numerous active substances. Naturally, therefore, it has been suggested that the different morphogenetic results of auxin action are due to a number of different hormones whose distri-

bution is in some way controlled by auxin. Thus when auxin is applied to the base of a cutting and causes the formation of roots there, it is suggested that it does so by mobilizing in some way a special root-forming substance. In the case of bud inhibition, auxin applied at the apex of the stem might inhibit the development of the buds lower down by mobilizing, towards the apex, the bud-growth substance, so that the lateral buds are deprived of it. There is, however, good evidence against this view. When auxin is applied directly to the lateral buds it ought to mobilize the bud-growth substance right at the bud, and thus promote bud development. But, both when the bud is on the intact stem and when the bud is isolated and growing in culture medium, such direct application only results in inhibition. Another explanation, in terms of a special bud-inhibiting substance, set free in the stem by the action of auxin, has been proposed by Snow, as a result of interesting experiments on the transport of inhibition. The evidence is again not decisive. The basis of bud inhibition may, of course, be simply that the response of buds to auxin differs quantitatively rather than qualitatively from the response of stems. To make my meaning clear I must point out that even stems and coleoptiles are inhibited in their elongation by auxin at very high concentrations. The rôle of concentration in these phenomena is of the greatest importance. The response of stems to a range of concentrations of auxin is really an optimum curve. Only within what may be termed the physiological range, that is, the range of concentrations normally present in the plant, does an increase in auxin lead to increased growth. Now the position of this optimum probably varies with different organs. In buds it may be very low, so that even physiological concentrations of auxin are in the inhibiting range. If this is so, very low concentrations should *promote* bud growth, and indeed there is good evidence that they do.

It was mentioned above that auxin also inhibits root

elongation. Here there is better evidence that the inhibition is a direct one, and that the optimum of the curve lies indeed at an extremely low concentration. Isolated roots in culture are strongly inhibited by auxin; exceptionally low concentrations, however, do somewhat accelerate root growth. The addition of vitamin B_1 in optimal amounts to the culture medium does not remove the inhibition. Furthermore, the addition of iodoacetate, which opposes the growth-promoting action of auxin on coleoptiles, also opposes the growth-inhibiting action on roots. There is thus every reason to believe that this inhibition is a direct one, and hence that one and the same substance at the same level of concentration may cause opposite effects in root and shoot.

Lastly, there are some peculiar facts which indicate that inherent quantitative differences in responsiveness are of importance in many growth processes. Thus in the induction of root formation in cuttings from seedling trees, lateral branches form roots more readily than the terminal shoot (auxin conditions being the same) although both are cut from the plant at the same point. In some trees, basal sections of the stem form roots more readily than apical sections (auxin conditions being again the same).

Another case is that of slit stems. When growing stems are slit longitudinally and immersed in auxin solutions, the two halves curve inward, the tissue layers near the epidermis elongating more than those near the center. After many unsuccessful attempts to ascribe this to various influences of the wound made on slitting, it has now, I think, become necessary to admit that the difference in growth is due to difference in sensitivity to auxin in the different layers of tissue. This has no clear connection with anatomical differences between the cell layers, since it is shown *par excellence* in the coleoptile, which is mainly undifferentiated.

The idea of "inherent" differences in responsiveness, whether quantitative or qualitative, of course raises as many questions as it settles, though from the morphologi-

cal and morphogenetic view-point it is a helpful idea. It may be suggested that the explanation is something like this. In order to cause growth, a hormone is only one of the factors needed. Sugars, nitrogenous substances, water and other factors are also essential. Differences in the rate of supply of these would bring about quantitative differences in response to the hormone. Physical differences in the structure of the cell walls will also control the nature of the response. Further analysis of some of these phenomena is already vigorously under way in a number of laboratories, and is uncovering some interesting new leads.

I have attempted to survey some of the morphological aspects of hormone action in plants, and to show you not merely that hormones do control plant development—which has been known for some years—but also something of *how* this control is exercised and the nature of the experimental attack on it. It is clear that the problems are very extensive, but on the other hand we do already possess, through our existing knowledge of the plant hormones, something like control over many processes of growth and development, and this is the first step towards their understanding.

PRELIMINARY ANALYSIS OF ACTIVITY OF THE CAVE CRAYFISH, *CAMBARUS PELLUCIDUS*[1]

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THE environment of caves and their inhabitants embrace one of the more interesting aspects of biology. The work of Banta, Jeannel, Eigenmann, of Packard and many others has served to open a vista of special speleological problems, the solution of which affect diverse phases of biological theory. Most cave problems require long experimentation or observation; accumulation of critical data on life histories, ecological interrelations, genetics and physiological adjustment is far from complete. Among the problems of caves, the activity of cave animals is of interest, and the following information on a typical cavernicole is presented herewith.

MATERIALS AND METHODS

The cave crayfish (*Cambarus pellucidus* Tellkampf) was used throughout the experiments to be described. In all, four collections, totaling 59 individuals, were made as shown in Table I.

TABLE I
COLLECTION OF MATERIAL

Date	Locality	Sex of specimens	
		Males	Females
March, 1935	River Styx, Mammoth Cave, Ky.	No. 1, 3, 4, 5	No. 2, 6, 7, 8, 9
November 19, 1936	River Styx, Mammoth Cave, Ky.	No. 10, 11, 12, 13 19, 20, 21, 22, Sn	No. 14, 15, 16, 17, 18 23, 24, 25, 26, 27
March 26, 1938	River Styx, Mammoth Cave, Ky.	No. 28, 29, 30, 37, 38, 39	No. 31, 32, 33, 34, 35 36, 40
November 11, 1938	McBride Cave, Ky.	No. 50, 52, 54, 55, 57	No. 51, 53, 56, 58

[1] This paper is the eighth in a program of the senior author on activity. The work was made possible by a grant from Northwestern University, and the authors are also obligated to Professors J. W. Buchanan and C. L. Turner, and to Mr. Schuyler Hunt, guide at Mammoth Cave, for many favors received during the investigation.

All the animals (Fig. 1) arrived at the laboratory in

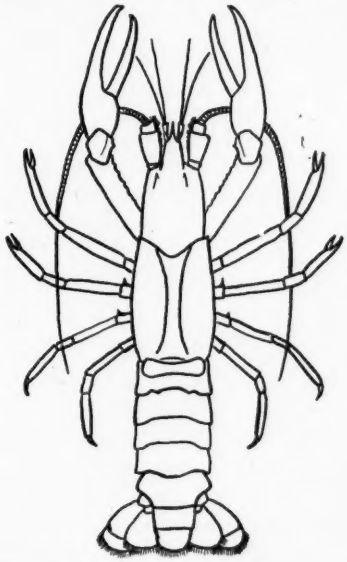

FIG. 1. *Cambarus pellucidus* Tellkampf, male, Mammoth Cave, Kentucky, from River Styx, March, 1935.

light-tight containers and were placed in a refrigerator. Later they were sexed, given numbers and divided among the several experiments. The species is very hardy and excellently suited for biological investigation. No unusual precautions have to be taken to insure a proper oxygen concentration and its food requirements appear to be satisfied by yolk of boiled egg. At least our animals lived under varying laboratory conditions for from six months to two and a half years, and during this time were fed every two weeks on egg yolk, each individual being given a piece of yolk about the size of a large pea. Where running tap water was not used, crayfish in quiet aquaria had a change in water every two weeks, about twelve hours after feeding. Under these conditions the species fed, moulted, regenerated lost parts and presumably remained in good physiological condition. Dur-

ing the experiments to be described the animals were exposed to a wide range of light intensity and temperature and tolerated these extremes. Their temperature tolerance is especially notable when one takes into account the great constancy of their natural physical habitat. Crayfish were frozen in blocks of ice, thawed out and placed in an experiment; others were placed in a beaker of water which was warmed over an electric stove. The water was warmed from 16° C. to 32.5° C. in fifteen minutes and at that point individuals were thrown into heat rigor; these animals were then placed directly into water at 16° C. again, and recovered in two minutes. These observations are given to substantiate the above statement concerning the merit of the species for biological investigation, and the temperature tolerance has been similarly tested by Putnam (*cf.* Packard, 1886, p. 125).

Two general methods were used in a study of activity of the species. The first of these involved no special apparatus, and was used in a study of the phototropism of the animal. In these trials the individual crayfish was placed in a Petri dish (8.25 inches diameter) of tap water. In some of the trials the water was allowed to fluctuate in temperature, in other trials the dish was placed in a water bath and the temperature controlled. A semicircular shield was fixed over the open top of the dish, in such a way that it could be rotated and bring any half of the dish into darkness or light. A light source from above, of known intensity, was then turned on and the movements of the animal, and time were charted until the individual came to rest at one portion of the dish. The intensity of light was measured in foot-candles by a Weston illuminometer, and the several results are reported later in this paper.

The second method was necessarily complex, in that a study of activity of the species, with respect to illumination, was attempted. Activity was recorded in all trials by objective equipment. This consisted of a modification of the Spencer ichthyometer (1929). A silk thread was

tied around the cephalothorax, usually between the second and third pair of pereiopods, and the free end of the thread looped over the end of a lever. This lever was delicately balanced by compensation weights at the other end, and worked in a vertical plane. Near the fulcrum, the lever carried a flexible recording stylus. Movements of the crayfish were transmitted via the thread, and subsequent movement of the lever, to the stylus which recorded the movement upon a two-inch wide strip of waxed paper, carried on an aluminum drum. The drum revolved once in twenty-four hours, by means of a Taylor clockworks, and moved 27 mm per hour. This recording equipment is sensitive to walking movements of the crayfish, leaving an excellent record of its activity through the twenty-four hours on the waxed paper. The lever must be compensated for each individual used, but beyond this adjustment needs little attention. The crayfish, so attached to the lever, were placed in small aquaria or battery jars. On plain glass the animal was unable to obtain traction, resulting in a great deal of abnormal activity due to the slight pull of the lever. On rocks, gravel or screening excellent traction was maintained and the great majority of trials were run with a circular piece of copper screening on the bottom of the battery jar to allow the animal to walk about easily. In experiments with the normal fluctuation of light and darkness, constant light and reversed illumination, a battery of four separate recorders was used, each crayfish isolated in a battery jar, and recording by lever to a separate drum and clockworks. These four jars were placed in a water table, surrounded by flowing tap water. This technique was varied by using animals in flowing water or quiet water with the temperature controlled, so that a wide variety of experimental conditions was available. For the trials in constant darkness, the crayfish were run one at a time. The recording system was essentially that described above, and the recording equipment and crayfish container placed in a light-tight, thermostatically controlled

cabinet (Fig. 2). Such trials could be run in quiet or

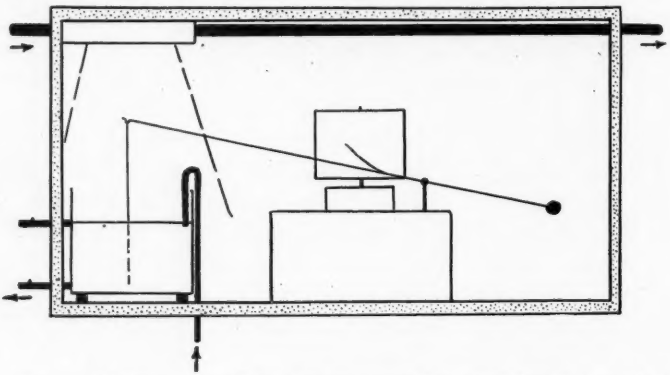

FIG. 2. Diagram of modified Spencer recording apparatus in insulated, light-tight, thermostatically regulated cabinet. See text for description.

flowing water, and if desired the animal could be illuminated, in which case light, passing through an uncovered port, was filtered through a water screen. Two types of dark cabinets were employed, differing in insulation and temperature control, and the several authors overlapped in the running of the various sets of trials so that a constant check could be maintained on the experimental conditions. All light intensities were measured by a Weston illuminometer, and this instrument was regularly checked against a second identical instrument which was returned to the manufacturers at intervals for rechecking. Both instruments were also checked against a standard lamp in the laboratory. These experiments are considered preliminary in that activity under various light intensities and conditions of illumination was studied, but holding light constant and varying the water temperature was not undertaken. The effect of temperature upon activity, therefore, was not investigated and this influence is to be examined in the future.

PRESENTATION OF DATA

Response to Light. Since the activity of the cave crayfish was to be studied under various conditions of illumi-

nation, it was desirable to ascertain whether the species was photonegative, photopositive or indifferent to light intensity. The eyes of *pellucidus* are vestigial. The eye-stalks are very short and nearly hidden beneath the rostrum, and of the same color as the rest of the adjacent integument. According to the work of Leydig and others (Packard, 1886), there is no facetted cornea, no crystalline lenses and no pigment in the eye-stalks but the optic nerves are present. Despite such a reduced optic equipment, the species is photonegative, but whether the response is a consequence of stimulation of the eye-stalks or of some other photoreceptor was not determined. It would be of interest in this regard to discover whether or not the species had a caudal photoreceptor as was reported of epigean crayfish by Prosser (1934) and Welsh (1934).

Using the method described, single crayfish were placed in the dish and half of the area exposed to light of a known intensity. Beginning with the animal in the illuminated portion, its movements were recorded on a time chart until a definite cessation of activity was observed. A typical trial is shown (Fig. 3). In all these trials the response of the animal was by wandering movements, into and out of the illuminated area. Such response is similar to the wandering movements of epigean species of crayfish in which both eyes are removed (Welsh, *l. c.*) and not by direct orientation. We believe that the light response of *pellucidus* is a trial-and-error response. Such behavior is to be expected in view of its constantly dark natural habitat and lack of well-formed eyes.

Seventy-two such trials were conducted, at light intensities of 18, 50, 100, 200 and 300 foot-candles, with and without temperature control, using nine different animals, and with both sexes represented in the sample. Only one trial was not markedly photonegative; in seventy-one trials the animal was definitely photonegative and came to rest in the shaded portion of the dish

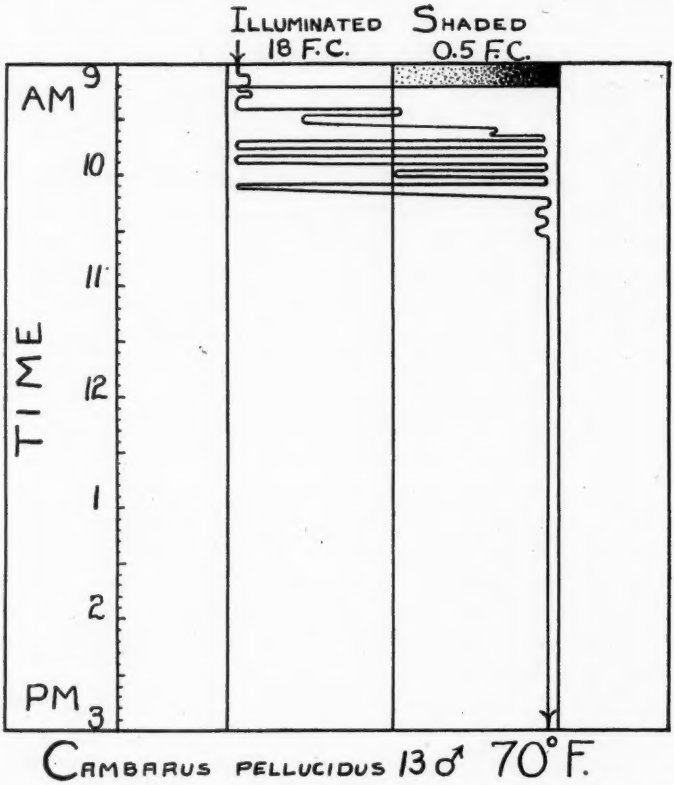

FIG. 3. Time-chart, showing typical response of a cave crayfish. The trial and error selection, with eventual photonegative position is usually attained within one to two hours.

(0.5 foot-candle) within one to two hours from the start of the trial. No animal came to rest in the illuminated area, and some times the final response was within thirty minutes. From such data it is clear that *Cambarus pellucidus* is generally photonegative, but that the response is not a direct orientation, but accomplished by the trial-and-error method.

Activity Pattern. Using the recording apparatus already described, the species was studied with respect to its total activity over the twenty-four hour period.

The following table summarizes our data on this subject (Table II). The eight columns give, from left to right, the date upon which each trial of twenty-four hours was started, the number of the experimental animal and its trial sequence, the time of start of trial, the type of illumination under which the trial was conducted, the condition of the water, the total number of activity units for the trial, and the percentage of nocturnal and diurnal activity for the trial.

The several types of illumination are fully explained in the table on illumination and activity (Table III). The last three columns in Table II need clarification since these columns hold the essential information. The term "activity unit" is an arbitrary activity standard adopted for the experiments recorded, and refers to a peak on the waxed-paper record. Thus, counting the peaks gives the total peaks or activity units per trial. When the record is divided into hours, then the number of activity units per hour is obtained. If the total activity units equal 100 per cent. activity for a trial, then the nocturnal units and diurnal units can be cast into the percentage of time the animal was active at night, and active at day, respectively.

Expressing the activity of *pellucidus* in percentage of time active per hour for a given twenty-four-hour trial allows a quantitative comparison of the activity in this species to be made with the percentage of time active per hour for different animals, of widely differing habitat and taxonomic position, in which different types of activity units and recording apparatus are used. In these activity trials the duration of "day" and "night" was arbitrarily fixed at between 6:00 A.M. to 6:00 P.M. for the day, and 6:00 P.M. to 6:00 A.M. for the night since the animal normally inhabits a totally dark habitat, in which there is no periodically illuminated portion of the twenty-four-hour cycle.

From an examination of these data, amounting to over four thousand animal experimental hours, it becomes

TABLE II

ACTIVITY OF CAMBARUS PELLUCIDUS
(April 16, 1935 to December 5, 1938)

Date	Animal and trial	Start of trial	Light type	Tap water condition and °F.	Total activity units (100 per cent.)	Nocturnal activity per cent.	Diurnal activity per cent.
1935							
iv. 16	No. 1–1	Noon	N–1	Quiet, 56	188	49	51
iv. 29	" 1–2	11:00 a.m.	"	" 56	428	49	51
iv. 30	" 1–3	Noon	"	" 56	232	53	47
iv. 24	" 2–1	3:30 p.m.	"	" 56	194	75	25
iv. 25	" 2–2	6:00 p.m.	"	" 56	373	93	7
v. 2	" 3–1	9:00 a.m.	KD	" 56	583	45	55
1935–1936							
xi. 22	No. 10–1	11:15 a.m.	KD	Running, 56	339	42	58
xi. 24	" 10–2	5:35 p.m.	"	" 56	263	47	53
xi. 25	" 10–3	6:25 "	"	" 60	170	46	54
xi. 24	" 11–2	5:40 "	"	Quiet, 70	592	57	43
xi. 25	" 11–3	6:25 "	"	" 70	248	53	47
xi. 28	" 11–4	1:50 "	"	Running, 62	536	47	53
xi. 29	" 11–5	4:00 "	"	Quiet, 70	192	57	43
xi. 30	" 11–6	8:00 "	"	Running, 55	373	73	27
xii. 2	" 11–7	9:40 a.m.	"	Quiet, 69	471	51	49
xii. 3	" 11–8	11:40 "	"	Running, 51	339	65	35
xii. 4	" 11–9	9:45 p.m.	"	Quiet, 66	327	54	46
xii. 7	" 11–10	2:10 a.m.	"	" 68	436	33	67
xii. 9	" 11–11	10:45 "	"	" 77	628	45	55
xii. 10	" 11–12	10:50 "	"	" 77	444	64	36
xii. 11	" 11–13	5:35 p.m.	"	" 73	303	47	53
xii. 12	" 11–14	12:20 "	"	" 75	310	65	35
xii. 13	" 11–15	6:00 "	"	" 73	327	69	31
xii. 14	" 11–16	6:40 "	"	" 75	466	53	47
xii. 15	" 11–17	7:00 "	"	" 75	263	63	37
xi. 28	" 12–1	1:50 "	"	" 69	709	70	30
xi. 29	" 12–2	4:00 "	"	Running, 58	1030	76	24
xi. 30	" 12–3	8:00 "	"	Quiet, 68	223	54	46
xii. 13	" 12–4	6:40 "	N–2	Running, 46	124	27	73
xii. 14	" 12–5	6:55 "	"	" 47	226	39	61
xii. 15	" 12–6	7:20 "	"	" 48	231	41	59
xii. 16	" 12–7	7:50 "	N–1	" 48	144	53	47
xii. 17	" 12–8	8:15 "	"	" 49	192	44	56
xii. 18	" 12–9	9:30 a.m.	KL–2	" 49	106	55	45
xii. 20	" 12–10	8:30 "	"	" 49	83	49	51
i. 13	" 12–11	11:55 "	"	Quiet, 71	168	66	34
i. 14	" 12–12	Midnight	"	" 71	210	60	40
i. 20	" 12–13	12:40 p.m.	"	" 51	185	23	77
i. 21	" 12–14	12:10 "	"	" 51	169	30	70
ii. 8	" 12–15	11:53 a.m.	"	" 51	170	50	50
ii. 9	" 12–16	11:55 "	"	" 51	65	61	39
ii. 10	" 12–17	11:50 "	"	" 51	89	26	74
ii. 11	" 12–18	11:45 "	"	" 51	45	65	35
ii. 15	" 12–19	9:52 "	"	" 48	36	31	69
ii. 16	" 12–20	10:00 "	"	" 51	44	31	69
ii. 22	" 12–21	10:00 "	N–1	" 50	48	40	60
ii. 23	" 12–22	Midnight	"	" 50	53	58	42
ii. 24	" 12–23	11:57 a.m.	RL–1	" 50	42	40	60
ii. 25	" 12–24	12:30 p.m.	"	" 50	17	90	10
iii. 1	" 12–25	11:30 a.m.	"	" 50	74	61	39
iii. 4	" 12–26	9:51 "	"	" 50	37	58	42
iii. 5	" 12–27	11:50 "	"	" 50	40	52	48
iii. 8	" 12–28	10:00 "	"	" 50	47	60	40
iii. 9	" 12–29	10:25 "	"	" 50	28	45	55
iii. 10	" 12–30	11:54 "	"	" 50	130	34	66
iii. 11	" 12–31	8:10 "	N–1	" 50	55	54	46
iii. 12	" 12–32	1:00 p.m.	"	" 50	62	47	53
xi. 28	" 13–1	1:00 "	"	" 66	49	53	47
xi. 29	" 13–2	1:00 "	"	" 67	91	73	27
xi. 30	" 13–3	1:00 "	"	" 67	57	66	34
xii. 1	" 13–4	2:25 "	"	" 68	87	63	37
xii. 2	" 13–5	2:50 "	"	" 69	129	73	27
xii. 3	" 13–6	5:00 "	"	" 69	174	78	22
xii. 7	" 13–7	2:45 a.m.	"	Running,	425	30	70
xii. 8	" 13–8	3:00 "	"	"	136	36	64

TABLE II—(*Continued*)

ACTIVITY OF CAMBARUS PELLUCIDUS
(April 16, 1935 to December 5, 1938)

Date	Animal and trial	Start of trial	Light type	Tap water condition and °F.	Total activity units (100 per cent.)	Nocturnal activity per cent.	Diurnal activity per cent.
xii. 16	No. 13– 9	7 : 55 p.m.	N–1	Running, 48	561	64	36
xii. 17	" 13–10	8 : 20 "	N–3	" 49	134	44	56
xii. 18	" 13–11	8 : 25 "	"	" 49	152	39	61
xii. 20	" 13–12	9 : 35 "	N–1	" 49	100	69	31
i. 2	" 13–13	12 : 15 "	N–3	" 52	148	67	33
xii. 2	" 14– 1	9 : 40 a.m.	KD	" 53	545	32	68
xii. 3	" 14– 2	11 : 40 "	"	Quiet, 69	432	67	33
xii. 4	" 14– 3	9 : 45 p.m.	"	Running, 55	789	41	59
xii. 8	" 14– 4	2 : 40 a.m.	"	" 60	352	54	46
xii. 9	" 14– 5	10 : 45 "	"	Quiet, 74	728	39	61
xii. 10	" 14– 6	Noon	"	" 74	383	49	51
xii. 11	" 14– 7	12 : 20 p.m.	"	" 74	432	26	74
xii. 12	" 14– 8	5 : 35 "	"	" 74	267	54	46
xii. 13	" 14– 9	6 : 00 "	"	" 74	518	74	26
xii. 14	" 14–10	6 : 40 "	"	" 74	528	54	46
xii. 15	" 14–11	7 : 00 "	"	" 74	263	63	37
xii. 2	" 15– 1	3 : 20 "	N–1	Running, 45	590	41	59
xii. 4	" 15– 2	8 : 45 "	"	Quiet, 68	207	61	39
xii. 5	" 15– 3	9 : 00 "	"	" 67	120	40	60
xii. 7	" 15– 4	2 : 45 a.m.	"	" 67	214	52	48
xii. 8	" 15– 5	2 : 55 "	"	" 67	140	42	58
xii. 9	" 15– 6	9 : 45 "	N–3	Running, 45	603	46	54
xii. 10	" 15– 7	11 : 00 "	"	" 42	410	28	72
xii. 11	" 15– 8	11 : 35 "	"	" 43	124	6	94
xii. 12	" 15– 9	6 : 20 p.m.	"	" 46	348	43	57
xii. 13	" 15–10	6 : 45 "	"	" 46	218	32	68
xii. 14	" 15–11	7 : 05 "	"	" 47	324	20	80
xii. 15	" 15–12	7 : 25 "	"	" 48	490	30	70
xii. 9	" 16– 1	9 : 45 a.m.	N–2	"	310	27	73
xii. 10	" 16– 2	10 : 50 "	"	" 42	115	45	55
xii. 11	" 16– 3	11 : 40 "	"	" 44	85	42	58
i. 21	" 25– 1	12 : 12 p.m.	KL–3	Quiet, 51	252	55	45
ii. 9	" 25– 2	10 : 55 a.m.	"	" 51	248	41	59
ii. 10	" 25– 3	10 : 50 "	"	" 51	43	100	0
ii. 11	" 25– 4	11 : 50 "	"	" 51	273	43	57
ii. 15	" 25– 5	9 : 50 "	"	" 51	142	46	54
ii. 16	" 25– 6	9 : 57 "	"	" 51	83	23	76
ii. 22	" 25– 7	Midnight	N–1	" 50	338	58	42
ii. 25	" 25– 8	12 : 35 p.m.	RL–3	" 50	199	53	47
iii. 4	" 25– 9	9 : 50 a.m.	"	" 50	58	56	44
iii. 8	" 25–10	10 : 00 "	"	" 50	54	50	50
iii. 9	" 25–11	10 : 30 "	"	" 50	37	20	82
iii. 10	" 25–12		"	" 50	445	35	64
iii. 11	" 25–13	1 : 03 p.m.	N–1	" 50	217	27	74
1938							
iv. 5	" 28– 1	Noon	N–2	" 51	140	50	50
iv. 6	" 28– 2	2 : 00 p.m.	KL–2	" 51	16	72	28
iv. 7	" 28– 3	1 : 30 "	N–2	" 50	406	87	13
iv. 11	" 28– 4	Noon	"	" 50	77	82	18
iv. 12	" 28– 5	"	KL–2	" 49	27	16	84
iv. 19	" 28– 6	"	N–2	" 51	43	76	24
v. 2	" 28– 7	"	RL–2	" 51	132	31	69
v. 4	" 28– 8	"	N–2	" 51	203	79	21
v. 9	" 28– 9	3 : 00 p.m.	"	" 53	145	82	18
v. 10	" 28–10	4 : 00 "	KL–2	" 53	101	45	55
v. 11	" 28–11	4 : 00 "	"	" 53	90	61	39
v. 12	" 28–12	4 : 00 "	"	" 53	77	67	33
v. 18	" 28–13	Noon	N–1	" 53	196	65	35
v. 19	" 28–14	"	RL–2	" 53	141	56	44
v. 23	" 28–15	"	N–1	" 53	151	77	23
v. 24	" 28–16	"	RL–2	" 52	141	68	32
v. 25	" 28–17	"	"	" 53	191	60	40
v. 26	" 28–18	"	"	" 53	149	47	53
v. 27	" 28–19	"	"	" 53	242	48	52
v. 28	" 28–20	4 : 00 p.m.	"	" 53	123	63	37

TABLE II—(*Continued*)

ACTIVITY OF CAMBARUS PELLUCIDUS
(April 16, 1935 to December 5, 1938)

Date	Animal and trial	Start of trial	Light type	Tap water condition and °F.	Total activity units (100 per cent.)	Nocturnal activity per cent.	Diurnal activity per cent.
iv. 5	No. 29– 1	Noon	N–2	Quiet, 51	241	38	62
iv. 6	" 29– 2	2:00 p.m.	KL–2	" 51	81	49	51
iv. 7	" 29– 3	2:00 "	N–2	" 50	109	51	49
iv. 10	" 29– 4	Noon	KL–2	" 50	190	6	94
iv. 11	" 29– 5	"	N–2	" 50	93	50	50
iv. 12	" 29– 6	"	KL–2	" 49	42	63	37
iv. 19	" 29– 7	"	N–2	" 51	35	62	38
v. 2	" 29– 8	"	RL–2	" 51	109	9	91
v. 4	" 29– 9	"	N–2	" 51	44	43	57
v. 9	" 29–10	3:00 p.m.	"	" 53	81	53	47
v. 10	" 29–11	4:00 "	KL–2	" 53	44	57	43
v. 11	" 29–12	4:00 "	"	" 53	27	62	38
v. 12	" 29–13	4:00 "	"	" 53	16	94	6
v. 15	" 29–14	Noon	"	" 53	32	24	76
v. 18	" 29–15	"	N–1	" 53	74	56	44
v. 19	" 29–16	"	RL–2	" 53	76	61	39
v. 23	" 29–17	"	N–1	" 53	102	75	25
v. 24	" 29–18	"	RL–2	" 53	30	50	50
v. 25	" 29–19	"	"	" 53	1 (4 p.m.)	0	100
v. 26	" 29–20	"	"	" 53	0	0	0
v. 27	" 29–21	"	"	" 53	114	38	62
v. 28	" 29–22	4:00 p.m.	"	" 53	87	28	72
iv. 20	" 32– 1	4:00 "	N–2	" 53	210	55	45
iv. 22	" 32– 2	3:00 "	KL–2	" 53	177	50	50
iv. 24	" 32– 3	Noon	"	" 53	85	56	44
iv. 25	" 32– 4	"	N–2	" 53	106	55	45
iv. 26	" 32– 5	"	"	" 53	129	68	32
iv. 27	" 32– 6	"	"	" 53	118	72	28
iv. 28	" 32– 7	"	"	" 53	198	75	25
iv. 29	" 32– 8	"	"	" 54	272	44	56
iv. 30	" 32– 9	"	"	" 53	265	37	63
v. 5	" 32–10	3:00 p.m.	"	" 52	185	69	31
iv. 20	" 33– 1	4:00 "	"	" 53	284	48	52
iv. 22	" 33– 2	3:00 "	KL–2	" 53	160	51	49
iv. 25	" 33– 3	Noon	N–2	" 53	85	86	14
iv. 26	" 33– 4	"	"	" 53	151	67	33
iv. 27	" 33– 5	"	"	" 53	122	73	27
iv. 28	" 33– 6	"	"	" 53	100	62	38
iv. 29	" 33– 7	"	"	" 54	157	64	36
iv. 30	" 33– 8	"	"	" 53	141	54	46
v. 5	" 33– 9	3:00 p.m.	"	" 52	101	73	27
v. 15	" 33–10	Noon	KL–2	" 53	170	44	56
v. 18	" 33–11	"	N–1	" 53	151	69	31
v. 19	" 33–12	"	RL–2	" 53	103	69	31
v. 23	" 33–13	"	N–1	" 53	85	54	46
v. 24	" 33–14	"	RL–2	" 53	83	57	43
v. 25	" 33–15	"	"	" 53	95	50	50
v. 26	" 33–16	"	"	" 53	84	49	51
v. 27	" 33–17	"	"	" 53	166	55	45
v. 28	" 33–18	4:00 p.m.	"	" 53	72	68	32
xii. 5	" 53– 1	2:00 "	N–3	" 52	461	54	46
xii. 5	" 56– 1	2:00 "	"	" 52	203	66	34

clear that the species has no twenty-four hour frequency of activity. In other words, its activity is arhythmic as far as day and night are concerned. This arhythmicity is brought out graphically in Fig. 4, where ten consecutive trials in constant darkness and temperature, and the average of these ten trials, are plotted against time.

TABLE III

ILLUMINATION-TYPE SUMMARY
(181 twenty-four hour trials: 4,344 animal hours)

Type number	Explanation	Number of trials	Average net gain over 50 per cent. activity
N–1	0.5 foot-candles 6 a.m.–6 p.m.; 6 p.m.–6 a.m. dark	34	7 per cent. Nocturnality
N–2	30.0 foot-candles 6 a.m.–6 p.m.; 6 p.m.–6 a.m. dark	34	8 per cent. Nocturnality
N–3	100.0 foot-candles 6 a.m.–6 p.m.; 6 p.m.–6 a.m. dark	12	10 per cent. Diurnality
KD	Total darkness	34	4 per cent. Nocturnality
KL–1	0.5 foot-candles all of trial	(no trials)	
KL–2	30.0 foot-candles all of trial	28	1 per cent. Diurnality
KL–3	100.0 foot-candles	6	1 per cent. Nocturnality
RL–1	N–1 reversed, 0.5 foot-candle during night; day dark, not more than 0.3 f.c.	8	5 per cent. Nocturnality
RL–2	N–2 reversed	20	2 per cent. Diurnality
RL–3	N–3 reversed	5	7 per cent. Diurnality

In a perfectly arhythmic species, the amount of diurnality would exactly cancel the amount of nocturnality; that is, the species would have 50 per cent. of its activity

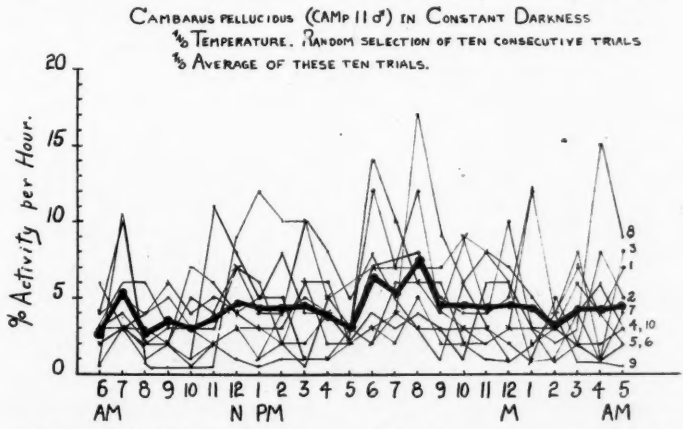

FIG. 4. Arhythmic activity of cave crayfish for ten consecutive twenty-four hour trials, with average of trials, in constant darkness and temperature. Note that each trial is independent of the other nine with respect to time active, and distribution of activity over the day and night.

at day and 50 per cent. of its activity at night. Therefore, if the gain in diurnality and gain in nocturnality be subtracted from each other, the resulting figure gives the net gain. From this method of analysis, a hypothetical species with 100 per cent. nocturnality would have a net gain of 50 per cent. on an actograph, while an arhythmic species would have a net gain of zero on the actograph. An analysis of the percentages given in Table II on nocturnal and diurnal activity of *pellucidus,* demonstrates very clearly the arhythmicity of the species (Fig. 5). Here we see that despite the variation in illumination,

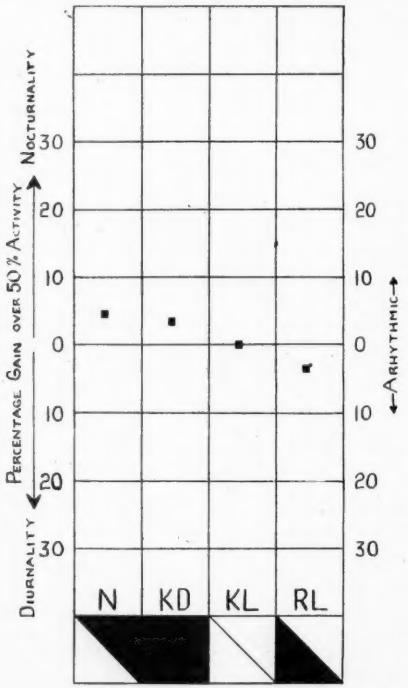

FIG. 5. *Actograph* of activity summary in cave crayfish. 181 twenty-four hour trials, 4,344 experimental hours, demonstrate that, in constant darkness, constant light of three intensities, or reversed light, and normal fluctuation of light and darkness, the average net activity for the species is near zero line, attesting to the arhythmicity of the animals. See Table III.

the over-all variation is from 5 per cent. nocturnality to 4 per cent. diurnality, with an average net gain for all trials under all types of illumination used, of 2.5 per cent. nocturnality; in other words, nearly perfect aperiodic activity for the species population. Individuals may be arhythmic, or nocturnal, or diurnal for a given trial, but there is no sustained activity at either day or night, and the trials tend to cancel each other.

In general, the animals tend to be more active at higher temperatures, as one would expect, although temperature was not directly varied to obtain precise information on this point. Thus, trials run under quiet water conditions averaged 133 activity units per trial at an average temperature of 12° C. and 362 activity units per trial at an average of 22° C. In its natural habitat, in the subterranean cave streams, the water temperature is approximately constant, usually between 14° and 15° C.

Activity was definitely increased by flowing water. Trials run in quiet water averaged 178 activity units per trial, while those in flowing water, at the same or lower temperatures, averaged 318 activity units per trial. In its natural habitat the rate of water movement is not great, in many of the pools and streams little or no movement of water is to be found. However, during vernal floods, with concomitant increase in amount and velocity of subterranean water, food would be more abundant. It is possible that at the spring of the year, therefore, with the possibility of higher water temperature and velocity, the observed experimental increase in activity at higher temperature and flowing water, might aid the animal to find food and locate niches which would not be otherwise accessible.

The natural food of the cave crayfish is very probably the small white cave isopod, *Caecidotea stygia,* and ecological equivalents. During flood, the available food supply is greatly augmented. Thus in Mammoth Cave, Kentucky, we have found epigean frogs (*Rana palustris*), forest snails (Polygyra) and stream insects (Chauliodes,

et cetera) after the flood water had drained away. This coincides with the data presented on flood-carried food by others (Hawes, 1939).

If the activity of the cave crayfish is analyzed on the basis of the Activity Index (A.I.) as formulated previously (Park, 1940a), in which nocturnality ranges from 1 to infinity, diurnality from 1 to 0, and arhythmicity at 1, the A.I. of *Cambarus pellucidus* for constant darkness works out at 3.4.

Possible Bearing of Data

It is well known that the cave environment is a constant one with respect to darkness, temperature, relative humidity and many other commonly measured factors. There is the possibility, of course, that more subtle influences such as twenty-four-hour periodicity in gravitational and in magnetic fields are operating upon cavernicoles, but in so far as the diel activity of the cave crayfish is concerned, the activity is arhythmic.

Here is a species of animal with an aperiodic activity, living in a relatively stable and constant environment. Elsewhere, the social, log-inhabiting beetle (*Passalus cornutus*) also has an arhythmic activity (Park, 1935, 1937) and inhabits a relatively stable environment within logs; certain social animals such as man and ants have apparently an arhythmic activity and live in a relatively stable social medium which is superimposed upon the periodic epigean conditions (Park, 1940); it would appear that fossorial mammals, which live in the relatively stable soil, are arhythmic in the sense that they repair damage to their tunnels at any time of the day or night (Hamilton, 1939). Thus, many species living in relatively constant habitats have more or less arhythmic activity patterns.

In contrast to this information, the overwhelming majority of species live in the periodic day-night epigean environment and have periodic activity patterns, whether exogenous or endogenous. Is it too much to assume that

we have here cause and effect? Survival value is certainly placed upon periodicity in activity in a sharply periodic habitat, and this periodicity of the earth surface has been continuing for a long time. Since a species can do one of three things, adjust, migrate or die, the data on activity would tend to indicate that cavernicoles, such as *pellucidus,* migrated into the more constant habitat of caves, by way of subterranean streams, and, if they had an endogenous periodism, this was eventually lost with decline in selection pressure. It may be that the slight nocturnality the cave crayfish appears to have (4 per cent. in constant darkness) may be a trace of an endogenous nocturnality, or this may be the result of insufficient trials in our experiment. At any rate, the majority of the epigean species of crayfish are nocturnal (Turner, 1926; Park, 1940).

At least there are many secondarily highly specialized species of animals living in caves, and some of these are of primitive stocks. Thus, among millipedes, the Polyxenidae are the most primitive living examples (J. W. Bailey, 1928) and agree with the fossil types in spinose pubescence. In Mammoth Cave, Kentucky, the milliped (*Scoterpes copei*) is secondarily adjusted in its eyelessness and white coloration, but bears the primitive spinose pubescence (V. Bailey, 1933). In Mammoth Cave evolution of cave animals must have taken place since the beginning of the erosion of the adjacent plateau, from one to four million years ago (late Pliocene to early Pleistocene). If evolution of these animals has taken place, that is, if the animals have evolved since entering caves, there is no reason to assume that such evolution has affected structure only, and not organismal activity pattern; and there is no reason to assume that any change in activity pattern has been in the direction of strengthening a diel periodicity which was adaptive on the earth surface.

In the cave crayfish another form of activity, namely breeding periodicity, seems to be little developed. One

female was observed "in berry" on June 26 and another carrying eggs in the middle of September. Mr. Hunt, an experienced guide at Mammoth Cave, tells us that he has seen a female with eggs in middle of September, 1937. In this instance the eggs were described as "clear" at time of capture. Another female was kept in a jar and observed by the guide, who said that between March 15 and April 15, 1938, the eggs turned a yellowish tint and the young hatched from the eggs during this month, left the female but returned to her a number of times. Such behavior checks with the observed activity of young epigean crayfish and lobsters. Bailey (1933) states that little is known of breeding in *pellucidus,* but that the eggs "are supposed to be laid during the winter and are carried . . . until they hatch." Application of the Van Cleave sampling technique might give us more information on breeding periodicity in this species, but the little information available indicates that the reproductive cycle, as well as the diel activity, is irregular when compared with phylogenetically related, surface-dwelling species.

This arhythmicity of activity would seem to be rather firmly fixed in *pellucidus.* Injection of physiological salt solution, distilled water blanks, or from 0.1 to 0.2 cc of boiled Ringers—*Cambarus blandingii, C. virilis* or *immunis* eye-stalk preparation, served to increase activity but not to change the arhythmic character of the activity of the cave crayfish, to any appreciable extent.

If descent from a nocturnal stock is any indication of "preadaptation" for the cave environment, *pellucidus* appears to qualify, as would the facultative inhabitation of caves by bats, and inhabitation by the cavernicolous carabid beetles (*Pseudanophthalmus*), millipedes, centipedes and certain other species. Recently Hubbs (1938) has presented evidence on the probable origin of cavefishes of Yucatan which he finds confirms the theory that cave animals have arisen from species "moderately preadapted to cave life."

SUMMARY

Under controlled experimental conditions, the cave crayfish, *Cambarus pellucidus*, was found to be generally photonegative and to have a species arhythmic activity pattern with respect to the twenty-four hour day-night cycle. The bearing of these findings on cave biology is discussed briefly.

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REVIEWS AND COMMENTS

EDITED BY CARL L. HUBBS

IN this section reviews and notices are given of current publications on general biology and of specialized works which have an important bearing in this general field. Emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution. No attempt is made to secure a complete coverage of the pertinent literature, or a uniform treatment. For contributions of major interest, however, interpretive reviews rather than abstracts are favored. During the first year there will be included reviews and notices of selected items which appeared in 1939 and 1940, as well as currently in 1941.

REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, University of Michigan, Ann Arbor, Michigan. All opinions are those of the reviewer.

The New Systematics. Edited by JULIAN HUXLEY. Oxford (and Toronto): Oxford University Press, 1940: i–viii, 1–583, 57 figs. $4.50.

TWENTY-ONE leaders in the field of modern systematics have here combined forces with Julian Huxley to demonstrate that systematics has attained its majority, and may now rank as a mature and worthy member in the brotherhood of biological subsciences. The volume is sponsored by the Association for the Study of Systematics in Relation to General Biology, in which too Julian Huxley has provided the leading hand. Again proving himself a worthy grandson of Darwin's Apostle No. 1, Julian Huxley sought far and wisely for chapter authors, and, in his "Introductory: Toward the Modern Systematics" saved the treatise from being a collection of rather unrelated (though generally excellent) essays. In this introduction the varied contributions of the coauthors, and of other workers, have been brilliantly synthesized into what is perhaps the soundest and best-written philosophy of systematics. All biologists should read at least Huxley's contribution, for he has deleted the repetitions that could not be avoided in such a treatise, has avoided pet theories and the occasional reversions to old systematics, and has sifted the mud out of the diction of the less skilled writers.

The twenty-one other contributions start and end strongly and hold up rather well through the middle of the large volume. W. B. Turill leads with a well-balanced essay on "Experimental and Synthetic Plant Taxonomy." Following with "Mutations and Geographical Variation," N. W. Timofeeff-Ressovsky provides very many examples to prove that mutations (of the simpler mendelian types) have often arisen in nature and at times have spread through populations and ranges. His interpretive thinking tends to be loose and dogmatic (or seems to be in a language foreign to the author), and he fails to consider the presumably multifactorial mutations that seem to have been much more potent in speciation—at least in vertebrates. Then C. D. Darlington expounds on "Taxonomic Species and Genetic System." Sewall Wright, in "The Statistical Consequences of Mendelian Heredity in Relation to Speciation," first loses most of us in mathematics beyond our comprehension, then proceeds with a presentation of his subject that is lucid, penetrating and provocative of research. Americans may well be proud also of their other contributor, H. J. Muller, who expands the "Bearings of the 'Drosophila' Work on Systematics," from the time of the dramatically pioneering work of Morgan, Sturtevant, Muller and Bridges down to the more concretely applicable current researches of Sturtevant, Dobzhansky, Timofeeff-Ressovsky and others.

In view of the more extensive and detailed treatments of the subject by the other authors, Lancelot Hogben's "Problems of the Origins of Species" appealed to the reviewer as relatively empty, but E. B. Worthington's "Geographical Differentiation in Fresh Waters with Special Reference to Fish" proved intensely interesting, in reference to the tie-up between ecology and speciation. Particularly significant is the very rapid speciation and adaptive radiation which, as Worthington has found, occurs in the absence of competition and predation, as though here again "nature abhors a vacuum."

"The Problem of Closely Related Species Living in the Same Area" is rather well and authoritatively presented by C. Diver, though too much stress is perhaps put on the results of the ecological survey of South Haven Peninsula. E. J. Salisbury contributes "Ecological Aspects of Plant Taxonomy" and W. H. Thorpe, a fine treatise on physiological races under the title, "Ecology and the Future of Systematics." In the first half of "Embryology and Taxonomy" G. R. de Beer treats his general subject with examples and references that seem strikingly few after the abundance supplied by Thorpe; the second half points out the bearings on systematics of the researches on relative growth (in which Julian Huxley has been the outstanding leader).

In treating "Palaeontology and the Taxonomic Problem," W. J. Arkell and J. A. Moy-Thomas rather overstress the mechanics and applications of systematic paleontological research, and in places appear a little dogmatic and conservative; but there is interest and value in the extended treatment of the contest between the conventional and simple "horizontal" classification and the perhaps hopelessly difficult "vertical" taxonomy based on apparent lineages with parallel specializations. In attempting a synthesis with the facts and interpretations provided by researches of other groups, most biologists will likely be left even farther in the air than the author (J. Ramsbottom) of "Taxonomic Problems in Fungi" (and it may have been wise that taxonomic problems in bacteria were left out of the volume). T. A. Sprague's rather generalized discussion of "Taxonomic Botany, with Special Reference to the Angiosperms," is followed by a brief chapter, "A Museum Zoologist's View of Taxonomy," by W. T. Calman, and then by J. S. L. Gilmour's "Taxonomy and Philosophy," which is largely just words. Primarily as related to the administrative problems of the British Museum (Natural History), John Smart treats "Entomological Systematics Examined as a Practical Problem."

In an outstanding contribution, "Polymorphism and Taxonomy," E. B. Ford treats in detail, with particular reference to insects, the taxonomic problems provided by the two or more phases which are not infrequently exhibited by one or both sexes in the same habitats (phases and geographical variations are perhaps too trenchantly distinguished, and no definite consideration is given to the relation of phases to mutations which show simple mendelian segregation and to those which exhibit approximately blended inheritance). H. H. Allan demonstrates clearly the significance of "Natural Hybridization in Relation to Taxonomy," though examples are drawn almost wholly from his own work on interspecific hybrids between the plant species of New Zealand. "The Origin and Behaviour of Cultivated Plants," by M. B. Crane, indicates points of significance in relation to speciation and systematics. In the final chapter—one of the most scholarly and significant—the prolific researcher N. I. Vavilov treats "The New Systematics of Cultivated Plants," and shows how thoroughly the characters of both domesticated plants and their wild relatives are harmonized with the climatic features of their respective environments.

Despite the thorough coverage of the subject from so many viewpoints—or perhaps because of this form of treatment—sufficient coordinated emphasis does not appear to have been given to such general subjects as the rôle of experiment in modern systematics; the principles of geographic variation; the analysis of subspecific intergradation; importance of different types of isolation in nature; and the rate of speciation.

The general agreement of the many authors is nevertheless very striking. The consensus of interpretation is that general systematics is becoming a comparative, interpretive, kinetic and often experimental field of research. It is made clear that many systematists are now welcoming, and profiting from, the aid that is furnished them by geneticists, cytologists, ecologists, physiologists

and specialists in other biological disciplines; and that systematics in turn is providing data and conclusions that are of value in the other subsciences. The distinction is breaking down between the systematist and the "biologist," and between the observer and the experimentalist, as also between the morphologist and the physiologist. A common front is being organized to attack central problems in pure and applied biology.

Multiple Human Births. By HORATIO HACKETT NEWMAN. New York: Doubleday, Doran and Co., 1940: i–xii, 1–214, 19 halftones and 5 line-drawings. $2.50.

TWINS—there is probably no single topic in biology which combines more popular interest with genuine scientific importance. It is therefore most fortunate that such a topic should have been selected for the first book in the non-technical science series now being issued by the American Association for the Advancement of Science. This book is "Multiple Human Births," by Professor H. H. Newman, well-known authority and pioneer in this branch of human biology. It is a well-balanced résumé of the technical treatise, "Twins," by Newman, Freeman and Holzinger, and represents the first popular book on this important subject.

For the general reader, one can recommend the present volume with much enthusiasm. Every one is interested in twins and "supertwins," as Professor Newman prefers to call the higher multiples. At least ten million people in this country are either twins themselves or are closely related by blood or marriage to a twin pair. Such persons will certainly find much of interest in what the author has to say about twin folk-lore, the types, causes and frequencies of multiple births, hereditary tendencies in twin production, the hazards of twinning, comparison of twins with single-born, Siamese twins, mirror-imaging, the Dionne quintuplets, etc. Such questions are concerned with twins *per se*. Professor Newman, however, is much more interested in what twins can tell us about the effects of heredity and environment in human beings in general.

The presentation of this latter problem has been quite skilfully blended with the more spectacular information already mentioned, so that even the most casual reader can not miss the logic and importance of twin investigations in the attack on the age-old problem of heredity and environment. He will learn that one dare not ask the question: Is environment or heredity more important?—except as he may ask it with reference to particular traits and capacities. He will also discover that his question can only be answered in a certain statistical way, namely, by expressing, for each trait, the proportional amounts of the total variation in the population which can be ascribed to differences in hereditary constitutions and environments. Finally, he will learn that such nature–nurture ratios are not universal constants, but apply to particular populations only; that either heredity or environment can be made to appear more "important" by merely selecting a group more variable in that respect. Such notions as these are very important because their answers are now recognized by biologists as supplying the most useful information which one can acquire in connection with a great many problems of plant and animal breeding and of human eugenics and euthenics. Moreover, it is in terms of just such generalities as these that the average person prefers to think along lines of heredity and eugenics. Unfortunately, even many genetics text-books fail to enunciate these principles clearly or give detailed illustrations of the methods of nature–nurture assay. The reviewer is therefore inclined to regard "Multiple Human Births" as a very important contribution to the educational literature on genetics.

Reviewing in Chapter I the facts, fancies, fallacies and fiction about twins, the author finds that primitive man, savage tribespeople, even our modern pater and mater, have never been quite able to decide whether the occurrence of twins constitutes an evil or a blessing. Biologists, on the other hand, are quite agreed that twinning is a very happy event, for they see in every pair of twins an unexcelled opportunity for probing some of nature's

most perplexing problems. A large share of the book is therefore devoted to biological and statistical considerations in the interpretation of twin similarities. Most of the material discussed represents the fruits of the author's own 25 years of study, a detailed analysis of 50 pairs of one-egg twins reared together, 50 pairs of like-sexed two-egg twins reared together, and 20 pairs (all that could be found in the entire United States) of one-egg twins reared apart.

Although the existence of one-egg twins, sharing completely identical hereditary constitutions, is easily seen to simplify the solution of the heredity-environment problem in several important ways, Professor Newman also calls attention to some complications introduced by the processes of twinning. One-egg or "identical" twins have prenatal environments which are far more unequal than those occupied by two-egg twins. There is usually an exchange of blood supply in one-egg twins which works to the disadvantage of one member of the pair. There is also an "asymmtery mechanism," operating most effectively in twins which separate relatively late in development, and tending to produce the same sorts of differences in the two whole individuals that would normally be shown by the two sides of the body. It is therefore not surprising that one-egg twin fetuses show greater disparity in size than do two-egg twin fetuses. Even at birth one-egg and two-egg pairs show about equal variation in this respect. Likewise, two-egg twins suffer the same hazards to a lesser degree. On the other hand, the author also feels that postnatal environments of twins reared together, especially one-egg pairs, are probably considerably more uniform than those encountered by single-born sibs, even apart from time and age considerations. For such reasons Professor Newman is inclined to suspect that most of the variability exhibited by one-egg twins reared together is due to prenatal environmental inequalities. In view of some of these complications it is somewhat difficult to follow the author when he con-

cludes that "the shares of heredity in determining differences among siblings, at least among pairs of the same sex (two brothers or two sisters), should be the same as those worked out for the same-sexed two-egg twins."

These and many other subtleties in the problem of twin comparison are discussed by the author and one becomes aware that, in spite of the unique advantage afforded by human asexual reproduction, the evaluation of heredity and environment is still a very knotty problem. Nevertheless, the reader will certainly agree that twins are extremely valuable in the study of human inheritance. He will probably also applaud the suggestion that society should begin to make far more extensive use of twins in physiological and educational research. Finally, one must commend the author for a very lucid, well-organized and entertaining account. The exposition is, on the whole, marked by a soundness and caution which is often difficult to achieve in a science book written for the non-scientist reader.

CHAS. W. COTTERMAN

Race, Sex, and Environment A Study of Mineral Deficiency in Human Evolution. By J. R. DE LA H. MARETT. London: Hutchinson's Scientific and Technical Publications (Distributed by Chemical Publishing Co., New York). 1935 (1940): i–342, 13 figs. $8.50.

THE author was led into this encompassing, philosophical treatise through his observations of the failure of high-bred domestic animals to survive or prosper on colonial soils deficient in minerals that are required in fast growth, high milk production and other functions. He thus came to appreciate what seems to be a cardinal principle in speciation—that the development and functions of a race are genetically modified to harmonize with the usual environment, so that for example high-nutrition types are evolved to make full use of bounteously available food material and low-nutrition races are adjusted to survive where certain essential food elements are deficient. The evolutionary consequences of deficiencies in

iodine and calcium in particular are theorized upon. There seems also to be a basis of true significance in the correlation between sexual differentiation and racial differentiation.

Thus there is something sound in the foundation of this treatise, which as a whole is a house of cards. Untested hypotheses are built up, one atop another, and each in turn is implicitly relied upon not only in itself but also as the foundation for further super-structure. Through wide reading the author has located many hypotheses for his structure, and when he visioned the application of his researches on domestic animals to the biology of man —including his sex problems, pathology, economics and wars—he went back to college and won his "B.S., Dipl. with Distn. Anthropology (Oxon)," as he tells us on the title page. Marett has a brilliant but runaway mind scarcely inhibited by critical judgment or patient test. His book illustrates the futility of any present-day attempt by one man to cover so large a sector of science.

Biology of the Vertebrates A Comparative Study of Man and His Animal Allies. Revised Edition. By HERBERT EUGENE WALTER. New York: Macmillan Co., 1939: i–xxv, 1–882, frontispiece, 738 figs. $4.00.

THIS is a revised and somewhat enlarged edition of a well-known textbook, in which comparative anatomy is extensively integrated with general biology and repeatedly pointed toward human biology. The student unless well led by his teacher will be distracted at the length and detail of this text; the working biologist will be disappointed at the sketchy treatment of his specialty: where and how to draw the line must be a growing problem. The book is bounteously illustrated, and overly copied archaic figures are fewer than usual.

Science in Progress. Second Series. Edited by GEORGE A. BAITSELL. New Haven: Yale University Press, 1940: i–xii, 1–317, figs. 1–129. $3.00.

THE publication of the Sigma Xi national lectures of 1939 and 1940 may be regarded as one aspect of a defense

mechanism against the rising Tower of Babel in science. The ten lectures, well and understandably written by eminent leaders in their respective fields, provide good examples of the progress that is being made along diverse paths in the different sciences, and help to demonstrate a unity of method.

In the first chapter, "The Experimental Alteration of Heredity," L. J. Stadler presents in nontechnical terms the results of modern researches which have so penetratingly attacked the problem of gene and chromosome mutations, with particular reference to mutations which have been experimentally induced. In treating "The Regulation of Plant Growth" the pioneering researcher F. W. Went leaves the lesson that the impressive progress in the study of plant hormones has resulted from the collaborative and mutually beneficial efforts of specialists in various disciplines: for example we may cite the demonstration that a gene-determined high oxidative level is correlated with the physiology of development in such a way as to lead to dwarfing. J. F. Fulton's "Experimental Studies on the Functions of the Frontal Lobes in Monkeys, Chimpanzees and Man" emphasizes the rôle of encephalization in the evolution of primate intelligence and hence in the origin of the human species. While explaining the cause of the "Mysterious Craters of the Carolina Coast," Douglas Johnson dramatizes "A Study in Methods of Research,"—simplifying the drama by some bright afterthoughts but providing a worthwhile contrast between the rôles of induction, deduction and critical analysis, in observational research. Alfred C. Lane's lecture on "How the Earth Shows Its Age," with its wide range of source references, is one of the most interestingly presented, particularly for biologists who are concerned with the rate of speciation and of evolution. The five other lectures treat the unconvincing hypothesis of an expanding universe, the marvelously interpreted cosmic rays, the motions of ions and proteins in electric fields, the ultracentrifuge and the timely subject of recent advances in aeronautics.

The Physical Sciences. By EMMET JAMES CABLE, ROBERT WARD GETCHELL and WILLIAM HENRY KADESCH. New York: Prentice-Hall, Inc., 1940: i–xvii, 1–754, frontispiece + figs. 1–300. $5.00.

THIS book is seemingly intended as the text for an orientation course in the physical sciences, and as a reference work for others who wish to have the diversity of all physical science encompassed in a single volume, in which the several disciplines are treated as "changing, growing fields that touch each other at a multitude of points." Human-interest applications are stressed.

HUMAN BIOLOGY IN AMERICAN UNIVERSITIES

IN the long view, looking toward the infinite future of man, all considerations of pestilence, famine, social breakdown, yes, and even war, are transcended by the development of human biology, the application of which is man's wise control of his own evolution. It might be expected that the universities—self-claimed centers of intellectual freedom and leadership—would long ago have fostered this relatively new science. Yet even to-day few universities offer a single course in human biology or include in either the teaching or research staff a single man basically and broadly trained in this fundamental field. Here we seem to see that the universities have been controlled by their other attributes—binding conservatism and unyielding tradition.

Signs are not wanting, however, to indicate an awakening of interest in human biology. The aspects of this science that have separately been developed—as medicine, psychology and even sociology—are broadening and becoming better integrated. Though still somewhat nebulous the nucleus of comparative sociology is taking form. Perhaps the most promising development in this direction lies in the recent advances of primate sociology, in which Yerkes of Yale has led the way. Human ecology too is being organized, though not uniformly with a strong biological background. At Harvard and throughout the land, physical anthropologist Hooton is making men

think in terms of human biology. Stanford is sponsoring a course on heredity and social problems (see review of Burlingame's book). Raymond Pearl of Johns Hopkins has passed on, but his work and the journal he established—*Human Biology*—live on. At Michigan, under the leadership of Dice, a Department of Human Heredity is being organized in the Laboratory of Vertebrate Genetics; Chas. W. Cotterman from Snyder's laboratory at Ohio State and the British human geneticist, L. S. Penrose, have been appointed to the staff, and a Heredity Clinic is being established in association with the Medical School and the University Hospital. In other institutions similar forward-looking developments are taking place. The biology of man may be in the ascendent.

SHORTER ARTICLES AND DISCUSSION

ABSENCE OF A TIME FACTOR IN THE PRODUCTION OF TRANSLOCATIONS IN DROSOPHILA SPERM BY X-IRRADIATION*

SAX[1] has reported that the proportion of "2-hit" chromosomal aberrations produced in Tradescantia microspores by a given dosage of x-rays becomes less as the same total dosage is given at lower rates for correspondingly longer periods. The proportion of these aberrations he finds to vary approximately as the 3/2 power of the total dosage if the intensity is kept constant, and as the square of the total dosage if the time of irradiation is kept constant. To explain his findings Sax advances the hypothesis that a broken strand will rapidly heal in the original combination unless some other broken and as yet unhealed strand exists in close proximity. Generalizing, he states[2] that "the frequency of x-ray induced aberrations induced in the Drosophila sperm must also depend on the time factor," and that "control of the time factor should also increase the exponent from 1.5 to 2.0 for the dosage-aberration curve in Drosophila."

This prediction has needed testing not only because of its bearing on the hypothesis, but also because of its bearing on certain other experiments, notably that of Muller and Mackenzie[3] involving a differential action of ultra-violet radiation, as compared to x-radiation, in the production of gene mutations and translocations in Drosophila sperm. The ultra-violet irradiation produced lethal mutations (presumably "single hit" aberrations) at a relatively low rate, which, on Sax's hypothesis, might conceivably explain the absence of translocations in the sample tested, since healing might occur between individual breakages.

To test Sax's prediction genetically, males of the Oregon-R strain of *Drosophila melanogaster* were irradiated[4] in 4 lots and their Y-bearing sperm tested for translocations involving either

* This manuscript was received for publication on March 2, 1940.

1 K. Sax, *Proc. Nat. Acad. Sci.*, 25: 225–233, 1939.

2 K. Sax, *Genetics*, 25: 41–68, 1940.

3 H. J. Muller and K. Mackenzie, *Nature*, 143: 83–84, 1939.

4 Maximar 220 x-ray tube used by courtesy of General Electric Company.. Dosage measurements kindly made by Dr. P. C. Aebersold at the University of California Radiation Laboratory.

two or all three of the following chromosomes: Y, II, III. Each lot was given a total dose of 5,000 r units at 220 KVP, but this dose was applied to the first lot in one half hour (temp. 20.5° C), to the second and third lots simultaneously over a period of 6 hours, 40 minutes (temp. rising from 21° C to 28° C), and to the fourth lot again in 30 minutes (temp. 28° C). Although the first and fourth lots showed a difference in percentage of translocations which happens to be in the direction expected on the basis of Mickey's[5] investigations (18.7 per cent. ± 2.25 per cent. for lot 1 at the lower temperature and 16.0 per cent. ± 1.83 per cent. for lot 4), this difference is statistically insignificant, and it is considered legitimate to combine these two lots in comparison with the second and third lots irradiated at intermediate temperatures.

The irradiated males were crossed to females containing the following markers: cinnabar brown (light eyes) in chromosome II, and ebony[11] (dark body) in chromosome III; sex itself marked the Y chromosome. The F_1 males were backcrossed to parental type virgin females, one male and two females being placed in each culture vial. Essentially similar tests have been described in several previous publications.[6]

The main results are given in Tables 1 and 2. The use of standard errors computed by the *pqn* formula is justified only if each sperm tested is a random sample of a population of sperm

TABLE 1

PROPORTION OF SURVIVING SPERM SHOWING TRANSLOCATIONS

Total dose r units	r units per min.	Total minutes	No. of sperm tested		Per cent. translocated (S.E. = $\sqrt{pq/n}$)
			Translocated	Normal	
5,000	167	30	117	564	17.2 ± 1.46 per cent.
5,000	12.5	400	136	665	17.0 ± 1.33 per cent.
Totals			**253**	**1,229**	**17.1 ± .98** per cent.

TABLE 2

Type of irradiation	No. of translocations involving the following chromosomes:			
	Y–II	Y–III	II–III	Y–II–III
Intense	21	14	74	9
Dilute	11	23	92	9
Total	**32**	**37**	**166**	**18**

[5] G. H. Mickey, *Genetics*, 23: 160, 1938.

[6] J. T. Patterson, W. Stone, S. Bedichek and M. Suche, AMER. NAT., 68: 359–369, 1934.

each of which has an equal and independent chance of being affected. Chi square tests suggest that these conditions are satisfied in the samples tested. Thus, chi square for the proportion of translocations among sperm from the 20 P_1 cultures is 12.95, which for 19 degrees of freedom has a P value greater than .8. Grouping by lots (1, 2, 3, 4) chi square is 1.52, degrees of freedom 3 and P greater than .5. The distribution of the 4 types of translocation among the 4 lots has a chi square of 7.56, 9 degrees of freedom, and a P greater than .5.

The data obtained are perfectly consistent with the proposition that no time factor exists under the conditions of the experiment. It can be stated with reasonable assurance that, even if a slight time factor does exist, the difference in the per cent. of translocations in the two populations can hardly amount to more than 3.5 per cent. This value is obtained by multiplying the standard error of the sum or difference of the two samples (1.97 per cent.) by 1.65 (which corresponds to a P value of .05, considering that the hypothesis requires a difference in a particular direction) and adding to the product the observed difference of .2 per cent.

Sax[7] records a drop from 17.3 per cent. to 7.0 per cent. when the time of irradiation is increased by a factor of 16. Consideration of his hypothesis makes it clear that a significant deviation should have been obtained in the present experiment (with a time factor of 13.3) if the postulated mechanism applies to Drosophila sperm unless the time of healing is very much greater than in Tradescantia microspores. The healing time would have to be nearly as long as or longer than that of the longer exposure (6 hours, 40 minutes), and in this event control of the time factor could not increase the exponent from 1.5 to 2.0 for the dosage-aberration curve in Drosophila, since the usual period of x-irradiation, in experiments not designed to test the time factor, is very much less than this value.

The ultra-violet irradiation in Muller and Mackenzie's experiment was given at a rate equivalent, in its production of gene mutations, to 30 r units of x-rays per minute and was complete in 20 minutes. It seems clear, therefore, that the time factor, if it exists at all, is wholly unimportant in that experiment.

It should be pointed out that the results here reported do not necessarily discredit Sax's general hypothesis, since the postulated healing process might reasonably be expected to occupy a

[7] K. Sax, *Proc. Nat. Acad. Sci.*, 25: 225–233, 1939.

much longer time in Drosophila sperm or even to await the resumption of developmental processes in the fertilized egg.

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Addendum: Since this article was accepted for publication Dr. A. Marshak has shown the writer a mimeographed abstract of a paper delivered by H. J. Muller at the Seventh International Congress of Genetics at Edinburgh, 1939, in which he reports that the same total ionization produces the same proportion of gross structural changes in Drosophila sperm whether the irradiation is delivered at .01 or 200 r per minute.

SEX RATIO IN DOMESTIC CHICKENS[1]

LANDAUER and Landauer (1931), in a summary of data from various workers, reported the sex ratio in domestic chickens to be 48.77 at hatching, including 67,993 chicks. Byerly and Jull (1935) stated that published reports on 6,864 dead embryos showed that 48.59 per cent. were males. These workers examined the sex of 17,989 embryos that had died after nine days of incubation and found 47.56 per cent. to be males. These data seem to indicate that embryonic mortality is significantly greater after the ninth day of incubation among females. If the primary sex ratio is 50–50, there must be a greater male mortality during the first nine days of incubation in order to give a sex ratio at hatching of 48.77.

Rhode Island Reds have been bred for high fecundity at the Massachusetts Agricultural Experiment Station since 1913. The sex has been recorded on all chicks at eight weeks of age. For the eight generations produced from 1933 to 1940, sex was recorded on 23,273 individuals and showed 50.85 per cent. males. These data are not in agreement with those of other workers. Since Landauer and Landauer (1931) have shown that there is a significantly higher mortality in male chicks up to eight weeks of age, it is difficult to account for the 50.85 per cent. males in our Rhode Island Reds at eight weeks. There is the possibility that in breeding for high fecundity the sex ratio may have been changed.

The sex ratio is not affected by date of hatch according to the

[1] Contribution No. 384 from the Massachusetts Agricultural Experiment Station.

studies of Lambert and Knox (1926), Horn (1927) and Lambert and Curtis (1929). The birds at the Massachusetts Station were hatched each year in four weekly hatches in March and four in April. These data show that at eight weeks of age 51.24 per cent. of the March chicks were males and 50.31 per cent. of the April chicks were males. Our data do indicate something of a tendency toward a higher sex ratio in early spring.

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CRITICAL COMMENTS ON THE THEORY OF THE INFLUENCE OF THE FLUVIATILE ENVIRONMENT ON THE SHAPE OF EARLY FISHES

THE theory that fishes originated in fresh water began with the well-known geologist, T. C. Chamberlin, forty years ago. Smith (1932), in a paper summarizing strong physiological evidence for the theory, states that, from conversations with paleontologists and geologists, he is aware that the idea has been received with skepticism. Ichthyologists are generally of the same opinion. Nevertheless, the theory is slowly gaining ground. It is not the purpose to consider here the pros and cons of this matter, but to discuss a cognate hypothesis, namely, the supposed effect of the river environment on the shape of early fishes.

Briefly, it was hypothecated that the fusiform shape of fishes is due to their origin in a fluviatile environment and was originally an adaptation to swift currents. This theory was advanced by Chamberlin (1900) with the theory of fresh-water origin and the two ideas have often been considered together, probably to the detriment rather than the confirmation of the theory of fresh-water origin, although the two were supposed to

bolster each other. Crucial evidence for or against the freshwater origin of fishes will come instead from the fields of paleontology and physiology. Although the theory that the streamlined form of fishes originated as an adaptation to river currents has not gained a great deal of credence, it was accepted by some well-known workers, namely Barrell (1916) and Lull (1922), and is mentioned in about half of the introductory texts on vertebrate zoology and evolution printed in this country and Great Britain during the past fifteen years.

Berry (1925, 1929) probably gave the best adverse criticism of the hypothesis, stating that certain theorists had the cart before the horse when they said that the environment moving by the early fishes determined their form, for in general it is the animal that moves through the environment. The ancient ichthyosaurs, as well as the modern cetacea, have all assumed the spindle-shape. Except for a few of the smallest porpoises, they are not and were not river animals.

The theory that river currents caused the early fishes to assume the spindle-shape was advanced without consideration of certain available facts. Other factors having some bearing on the question have come to light in the intervening years. Rivers are not solid walls of water, traveling at a uniform speed and direction at a given cross-section, as in a flume. The configuration of the bottom and banks and the bends in the streams cause backwashes, deflections and eddies. In swift-water sections of mountain streams, air bubbles and leaves can be seen rushing upstream for short distances at certain places. For thousands of years salmon have been able to lay their eggs in fast water, because the trench they dig causes the water to flow upstream in the trench and the eggs are not washed away before they can be covered with gravel (Hobbs, 1937). It is probable that in climbing such chutes of white water as Salmon Falls, on the South Fork of the American River in California, salmon take full advantage of deflections in the roaring current in addition to using their great strength.

Eddies are common in large rivers. Shanty-boatmen on the Mississippi skilfully take advantage of these currents to travel effortlessly upstream, where they are present. Several years ago these men told the writer that fish travel in trails through the water, and this seems to be common knowledge with them. The observation is plausible, for fishes taking advantage of more or less permanent configurations of current flow would naturally

travel or appear to be traveling in trails. Furthermore, the fact that fish apparently travel in trails in aquaria has recently received attention from zoologists in this country and England.

Even the best known current breasters, the salmon and trout, take advantage of slack currents behind rocks and pools. It is a matter of elementary knowledge with stream biologists that sections of streams with a small amount of pool space will support only a small population of trout (Hubbs, Greeley and Tarzwell, 1932). The fish do not stand out and breast the current, but lurk for long periods in the quiet waters nearby. This is true of all fishes of fast streams. Stream biologists have arbitrarily defined four types of pools (Davis, 1938), the first and smallest being the kind that form behind boulders in rapid water. It is here that the fishes of fast streams often ensconce themselves. The riffle-sculpins, (*Cottus* spp.), of the northern fresh waters, often live in the swiftest, white water of mountain streams These animals are not ideally spindle-shaped. Instead, they are flattened, large anteriorly and attenuate behind, being the shape of half a streamlined solid. They are fitted to withstand the rushing water, while pressed down against the bottom. Nevertheless, they take full advantage of the minute pools and eddies behind the boulders of their torrential home.

Interesting evidence of the deflection of water currents by boulders was formerly present at the Rucka Chucka Debris Dam site, on the Middle Fork of the American River of California. Huge, natural, block-like, besalt boulders constricted the stream to a narrow channel. The upstream faces of the boulders were worn smooth, while the wall-like, downstream faces, at right angles to the stream, were pitted with numerous depressions, some of which had been worn to the pot-hole stage; all caused by water currents holding sand and gravel against the downstream faces and scouring them.

The brook lampreys, the spoonbill and the large male catfish inhabiting American rivers do not have the ideal spindle-shape. The writer pointed out (Gunter, 1938 a, b) that such nonstreamlined, weak swimmers as the blue crab, the American broad sole and the stingaree are known to invade rivers with strong currents, for over one hundred and fifty miles. Further search (unpublished manuscript) has shown that in North and Middle America other such non-fusiform, sluggish swimmers as four pipefish, two sawfish, three achirid soles, two pleuronectid flatfish

and two blowfish can invade rivers. The lampreys are not powerful swimmers, but four species travel up swift rivers of this continent. Smith (1936) listed four sawfish and thirty-eight rays from rivers of the world. These fishes are sluggish and flattened.

Hubbs (1940) has found that forms living in steep-grade creeks avoid riffles and live in pools and quiet pockets around boulders, and are therefore less fusiform than fishes in large, open rivers. If the fluviatile environment caused fishes to acquire the fusiform-shape, then as swiftness of the water increased the shape would be perfected. This is contrary to what is found, as is shown by the work of Hora (1927). In ponds and sluggish streams of India fishes are fusiform. In swift waters they are less so, being more cylindrical, and in torrents they are depressed and in some cases almost leaf-like. The tail becomes very muscular, to enable the fish to dart about from one rock to another. It is clear from this and other observations that as the waters become not only swift, but very turbulent, the fishes forsake the upper water levels and become creepers and crawlers with the insects, which they come to resemble in shape, and some of them even develop adhesive organs. Here, and here only it seems to the writer, can we postulate a modification of the shape of fishes brought about by river currents; and the modification is not in the direction of a fusiform-shape, but is rather a modification of that shape by progressive flattening, until in the extreme the leaf-like form, mentioned by Hora, is achieved.

Chamberlin's statement that marine fishes do not attain the spindle-shape as consistently as river species, needs qualification and is to a certain extent erroneous. The shapes of marine fishes are more diverse than those of fresh-water fishes just as their ways of life are more diverse, but no fluviatile fish has developed the elegant, fusiform-shape of many of the marine fishes, such as the mackerel, which can not breathe unless swimming through the water at a fairly rapid speed (Hall, 1938).

From these facts it is concluded that the fusiform-shape is not necessary for river fishes and conversely that river currents do not cause fishes to take on that shape, for the current can be circumvented and weak swimmers can invade rivers. They are enabled to do this by virtue of the condition that no river current flows evenly, but has many deflections and temporary reversals. The theory that the spindle-shape of fishes is a result of their fluviatile origin is, therefore, based on a fallacious assumption and is completely untenable.

It should be pointed out that this conclusion is not contrary to the theory of the fresh-water origin of fishes. Chamberlin's early fishes were an armored, "panoplied host" and not fully fusiform (Gregory, 1928). Chamberlin's critics were quick to note that his early, supposedly fresh-water fishes did not have the shape which he said the river environment gave. According to the argument advanced here, the early, benthic, non-fusiform fishes could have been successful inhabitants of rivers as well as of base-level swamps.

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